INFLUENCE OF THE QUARANTINE PEST *ALEUROCANTUS SPINIFERUS* (QUAINTANCE, 1903) (HEMIPTERA: ALEYRODIDAE) ON POMOLOGICAL AND PHYSICOCHEMICAL PROPERTIES OF *CITRUS UNSHIU*

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Abstract. The orange spiny whitefly, *Aleurocanthus spiniferus* (Quaintance, 1903) is a new quarantine pest in the territory of the Republic of Croatia that can cause significant problems in citrus production. The aim of this study was to determine the impact of the pest on the pomological and physicochemical characteristics of fruits and mineral composition of *Unshiu* leaves. The research was conducted in the private orchard of *Unshiu* 'Owari'. Heavy pest infestation was found in the plantations, which usually colonises the underside of the leaves. By sucking the plant sap, pests secrete abundant honeydew, which is inhabited by sooty fungi, and ultimately leads to reduced respiration and photosynthesis of the plant. Pest infestation significantly affected fruit pomological (weight, fruit height and width) and physiochemical properties (total acids, soluble dry matter, vitamin C and total phenols). Analysis of variance revealed a statistically significant difference between the studied characteristics of uninfested and infested fruits. It is an economically important pest that represents a major phytosanitary risk for agricultural production in Croatia, especially for citrus fruits, considering that citrus fruits represent the main fruit and export crop in Croatia. Continuous pest monitoring and agro technical and chemical measures attempts are being made to prevent its further spread to non-infested areas.
Keywords: *mandarin, fruit, leaf, whitefly, mineral content*

Introduction

In the last century, citrus cultivation flourished in the Neretva Valley thanks to the establishment of the agricultural and industrial complex "Neretva" in Opuzen (PIK Neretva) (Gatin, 1978; Kaleb, 2014; Marić and Paladin, 2017). Even today, the Neretva Valley is the largest and economically most important citrus area in Croatia. According to the Central Statistical Office of the Republic of Croatia (CBS, 2021), the total area under citrus cultivation is 2,096 ha, of which 1,612.89 ha are in Dubrovnik-Neretva County (APPRRR, 2021). The most important group for our growing area is Satsuma mandarin or *Citrus Unshiu* Marc. (Bakarić, 1983). This fruit species is one of the most cold-resistant citrus (Krpina, 2004) and is grown mainly in central and southern Dalmatia

(Bakarić, 1983). In nature, *Unshiu* is a dwarf stem, the canopy and shoots have distinct nodules and internodes and do not have spines. There are leaves on the nodules, and buds are located in the leaf axils. *Unshiu* develops a flower that is similar in structure to the flower of other citrus fruits, but differs in size, colour, fragrance, and function. Like other citrus, it has a hermaphrodite hypogenic flower, that is, an overgrown pistil. Due to these degenerative changes, *Unshiu* mandarin develops a parthenocarpic fruit (Bakarić, 1983). The fruit is a modified berry known as a hesperidium, usually elliptical-round, and consists of the rind, i.e., the pericarp, and the pulp, i.e., the endocarp. The rind accounts for about 30% of the fruit mass and consists of two parts, the outer flavedo and the inner albedo. The peculiarity of *Unshiu* lies precisely in the rind, which is very easy to separate from the rest of the fruit and, unlike other citrus fruits, is quite elastic (Skendrović Babojelić, 2009). Ripe, healthy fruits are rich in a variety of compounds that strongly influence the taste characteristics and organoleptic quality (Gattuso et al., 2007). According to Bakarić (1983), the chemical composition of mandarin fruit is the most diverse composition of organic and inorganic compounds, vitamins, minerals and organic acids. In Dubrovnik-Neretva County, the most common varieties of mandarin Unshiu are: 'Kawano Wase', 'Chahara', 'Zorica Rana' and 'Owari' (Marić and Paladin, 2017). Nitrogen (N), phosphorus (P), and potassium (K) are considered the most important macroelements for tree growth and yield. Nitrogen (N) is a component of enzymes, proteins, amino acids, nucleic acids, photosynthetic pigments, and other important compounds (Zekri and Obreza, 2013). High concentrations of nitrogen are found in young leaves, shoots, and buds. Another important element is phosphorus (P). The highest concentration of phosphorus (P) is found in the young plant parts, flower and seeds, and it plays a role in cell division and enlargement (Zekri and Obreza, 2013). Therefore, the plant stops growing when phosphorus concentration is too low. Potassium (K) improves plant health, disease resistance, and tolerance to nematodes and insects. When potassium is deficient, the rate of photosynthesis in plants drops sharply. The consequences of potassium deficiency are lower yields and poorer fruit quality, which occur before the symptoms of potassium deficiency are visible on the leaves (Zekri and Obreza, 2013). Apart from macro- and microelements, fruit quality and ripening are influenced by numerous biotic and abiotic factors. In many countries where citrus fruits are grown, including the Republic of Croatia, the most common harmful organisms, such as the Mediterranean fruit fly (*Ceratitis capitata* Wiedemann, 1824) or numerous pathogenic fungi or viruses, can occur, affecting the production and sale of citrus fruits. Their presence, spread and harmfulness depend on production methods, plantation maintenance and climatic conditions. In addition, the presence of a quarantine pest whose harmfulness is of great economic importance has been detected in some EU countries; these organisms are systematically monitored in the European Union and phytosanitary measures are taken to eradicate or prevent their spread. Of the quarantine pests that are dangerous to citrus, only the Orange spiny whitefly, *Aleurocanthus spiniferus* (Quaintance, 1903), has been recorded in Croatia. This polyphagous pest attacks numerous plant species from up to 38 different families (El Kenawy et al., 2014). In 2008, this pest was detected in southern Italy in the district of Lecce, whereupon this species was transferred from the European and Mediterranean Plant Protection Organization A1 (EPPO A1) to the EPPO A2 list (Porcelli, 2008). The pest causes direct damage by sucking plant sap on the undersides of leaves and indirect damage by abundant secretion of honeydew inhabited by soot fungi (Gyeltshen et al., 2005). Heavy infestations result in leaf drop and reduced fruit set (Cioffi et al., 2013). In Croatia, the species *A. spiniferus* was first detected in 2012 on ornamental

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orange seedlings (*Citrus x aurantium* L.) and subsequently successfully eradicated. Then, in 2018, the presence of an already established population was detected in Dubrovnik-Neretva County. In addition to Croatia and Italy, the pest has also been confirmed in Montenegro (Radonjić et al., 2014), Greece (Kapantaidaki et al., 2019), and Albania (Šimala et al., 2019; Nugnes et al., 2020). The species has acclimated to confirmed sites, spreading to new host plants and causing significant damage, especially in citrus orchards (Cioffi et al., 2013). Its spread poses a major phytosanitary threat to the production of other agricultural crops. The aim of this study was to investigate the impact of the quarantine pest *A. spiniferus* on the pomological and physicochemical characteristics of mandarin fruits and on the mineral composition of leaves in Dubrovnik-Neretva County.

Materials and methods

Object of the research

The research was conducted in a private mandarin orchard in Konavle municipality in Vitaljina (N42'37'39 E18'10'48), Croatia. The plantation is 0.05 ha and 18 Satsuma mandarin (*Citrus Unshiu* Marc.) of the varieties 'Owari' and 'Kawano Wase' were planted. Themandarin orchard is 35 years old, faces southwest and is located at 144 m above sea level. The distance between the trees is 4x4 m, and the canopy of the trees is dense and opaque. In the mentioned plantation, *A. spiniferus* was detected for the first time in 2018, and its population had already become established.

Variety studied

Variety *Citrus Unshiu* Marc. 'Owari' is the most commonly commercially grown variety. Satsuma mandarins grafted onto a trifoliate orange (*Poncirus trifoliata* L.) can withstand temperatures as low as -10 °C (Harrison et al., 2013). Leaves are green, one-pointed, narrow and toothed, and their surface is smooth (Bakarić, 1983). The fruits are orange, appear on annual shoots, almost seedless, and easy to peel. The outer part of the fruit or flavedo is coloured and inside there are numerous oil glands containing essential oils, acids, minerals, calcium oxalate crystals and many other substances (Bakarić, 1983). Mokbel and Hashinaga (2006) state that in citrus, the flavedo contains the greatest amounts of ascorbic acid, phenolic compounds, fiber, and other elements. The inner part of the rind or albedo is white, reticulate, and rich in cellulose, hemicellulose, lignin, pectin, and phenols (Bakarić, 1983). The albedo extends beneath the cells of the oil glands to the lobes of the fruit. The peculiarity of the albedo lies in its spongy structure (Skendrović Babojelić, 2009).

Harvesting and preparation of samples

The fruits of Satsuma 'Owari' were harvested in January 2021 at the stage of optimal ripeness. A total of 20 infested fruits (*Figure 1a*) were randomly harvested from two trees within the infested plantation. Uninfested fruit (20 fruit) were harvested from mandarin trees of the same cultivar located approximately 400 m away where no *A. spiniferus* infestation was detected (*Figure 2*). Fruit samples were analysed immediately after harvest in the laboratory of the Department of Mediterranean Plants, University of Dubrovnik.

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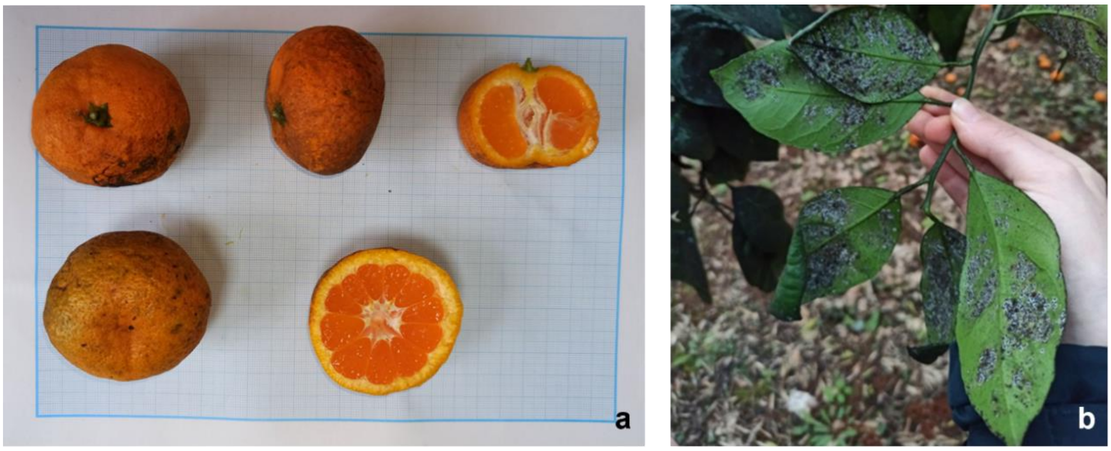

Figure 1. *Mandarin fruit covered with Capnodium spp. (a) and A. spiniferus on the underside of a mandarin leaf (Citrus Unshiu Marc.'Owari ') (b) (Photo: Paladin Soče, 2021.)*

Figure 2. *Map of the study region and the location of sampling (Google earth)*

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Sampling of leaves

Infested (*Figure 1b*) and uninfested leaves were harvested in October 2020. The leaf sample consisted of 50 leaves harvested from the central part at a height between 1.5 and 2 m on all 4 sides of the tree (north, south, east, west). Uninfested leaves were harvested from mandarin trees about 400 m away, where no infestation was detected. The fruit and leaf samples were collected from the same trees. After the leaves and fruits were harvested, they were packed in labeled PVC bags and transported to the laboratory of the Department of Mediterranean Plants, University of Dubrovnik, where further analyses were performed. To minimize errors, one person was responsible for laboratory analyses, while another person was responsible for the pomological description.

Laboratory analysis of the samples

Analysis of the fruits

The description of the pomological characteristics of the Satsuma fruits was done according to the International Plant Genetic Resources Institute (IPGRI) citrus descriptor with numerical scale of 1-10 (1 representing the lowest and 10 the highest rating). The following characteristics of the fruit were described: Fruit appearance and color, albedo colour, fruit size, fruit flavour, fruit axis filling, presence of seeds. After description, fruits were subjected to physico-chemical analysis and the following characteristics were studied: Fruit weight, height and width, fruit shape index, areola diameter, soluble solids content, total acids, citric and malic acid content, ash, total phenols, ascorbic acid - vitamin C and pH. Fruit weight was measured using an analytical laboratory balance (ABS 220-4, KERN & Sohn GmbH, Balingen, Germany) and expressed in grams to two decimal places. Fruit dimensions (height, width, and areola diameter; mm) were measured with a hand scale (PRO + 1930, BGS Technic, Wermelskirchen, Germany). Fruit shape index was calculated from the measured data as the ratio between fruit height and width. Soluble dry matter was read directly on a digital refractometer (OPTi Digital Brix 85, Bellingham + Stanley, Tunbridge Wells, United Kingdom) and expressed in degrees Brix. The pH was determined using a calibrated pH meter (Seven Easy S20, Mettler Toledo, Columbus, Ohio, USA). Citric and malic acids were determined by the enzymatic method (Mollering, 1989). Commercially available enzyme kits for malic acid (K-LMAL 06/17, Megazyme, Co.Wicklow, Ireland) and for citric acid (K-CITR 04/20, Megazyme, Co.Wicklow, Ireland) were used for the result. Results were recorded using a spectrophotometer (UV-1600PC, VWR, Radnor, Pennsylvania, USA) and expressed in g/L. Total acids were determined by potentiometric titration of the sample with 0.1 molar sodium hydroxide solution using a titrator (Easy plus, Mettler Toledo, Columbus, Ohio, USA) and expressed in g/L of citric acid. The ash content was determined by the standard method of annealing a sample of mandarin at a temperature of 500 °C until a homogeneous mass was obtained, after which it was cooled and weighed on an analytical laboratory balance (ABS 220-4, KERN & Sohn GmbH, Balingen, Germany) to constant mass. The ash content is expressed in g/100 g. Vitamin C was determined by the iodometric method of titration with iodide solution according to Silva et al. (1999). The vitamin C content is expressed in mg/100 g and calculated according to the following formula (*Eq.1* - Iodometric titration method with iodine solution for determination of vitamin C).

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$$\frac{x\ ml\ of\ iodide\ solution\ used\ for\ sample\ titration}{X\ ml\ vitamin\ C} = \frac{X\ ml\ osolution\ of\ iodide\ used\ for\ titration\ of\ stand.solution\ of\ vit.C.}{0.250\ g\ vitamin\ C} \quad \text{(Eq.1)}$$

Total phenols in the mandarin sample were determined spectrophotometrically using the method of Ough and Amerine (1998). The method is based on the color reaction of phenol with Folin-Ciocalteu reagent and measurement of the resulting color intensity at a wavelength of 765 nm. The results are expressed as mg of gallic acid equivalent (mg GAE/g).k

Analysis of the leaves

In the infested plantation, 50 leaves were harvested and carefully examined to prevent transmission of the adult stage of *A. spiniferus*. Leaves from uninfested trees were harvested on the same day. Leaves were transported to the laboratory in PVC bags. At the laboratory, they were washed in 70% ethyl alcohol to kill all live stages of *A. spiniferus*. The leaf samples were then dried in a muffle furnace (LE4 / 11 / R6, Nabertherm, Lilienthal, Germany) at 70 °C (24 h), ground to a powder in a porcelain mortar, sieved through a sieve with round holes diam. 2 mm, and stored in a refrigerator until analysis. A total of 10 g dry weight of the leaf sample was used for analysis. The following parameters were determined in the mandarin leaves: Total nitrogen, readily available potassium and phosphorus. The Kjeldahl method (AOAC, method 978.04) was used to determine total nitrogen. Analysis of total plant nitrogen consists of degradation, distillation (UDK 129, Scientifica VELP, Usmate Velate MB, Italy) and titration with 0.1 molar hydrochloric acid. Results are expressed as percentage of total nitrogen (%). Determination of readily available phosphorus and potassium by Egner method Al: The sample is extracted with a dilute Al solution for 4 hours at room temperature. The obtained phosphorus content of the extract was determined spectrophotometrically (UV-1600PC, VWR, Radnor, Pennsylvania, USA) and the potassium content was determined flame photometrically (Model 360, Sherwood Scientific Ltd, Cambridge, UK). The results were converted and expressed as %.

Statistical data analysis

A total of 20 uninfested and 20 infested fruits and 50 uninfested and 50 infested *Unshiu* mandarin leaves were harvested from a total of 4 randomly selected trees. All laboratory analyses were performed in three replicates. One-way analysis of variance (ANOVA) was used to compare the analysed characteristics of healthy and contaminated Unshiu mandarin leaves and fruits, and the mean values were compared using the Fisher LSD test in the statistical program SAS ver 9.4 (SAS / STAT, 2013). In addition to the results, the tables show different letters indicating significant statistical differences between uninfested and infested mandarin leaves and fruits at $P \leq 0.0001$. The average deviation of the results from the mean for each studied trait with the standard deviation values is also indicated.

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Results and discussion

The results of the description of pomological properties and physicochemical analyses of uninfested and sooty mold infested mandarin fruits are presented in *Tables 1-3*. Mineral composition of uninfested and infested mandarin leaves is presented in *Table 4*.

Table 1. *Evaluation of the general appearance, size and color of the fruit, the color of the albedo and the taste of the fruit (IPGRI,1999)*

Characteristics	**Uninfested fruits**		**Infested fruits**	
	Description	Mark	Description	Mark
Fruit appearance	Good	8	Bad	3
Fruit colour	Orange	9	Orange covered with sooty fungi	2
Albedo colour	Pale orange	5	Pale orange	5
Fruit size	Middle	7	Small	3
Fruit flavour	Sweet and very juicy	9	Juicy and sour	4
Fruit axis filling	Hollow	5	Hollow	5
Presence of seeds	No	10	No	10

According to the subjective description of the external appearance of the fruits, the uninfested fruits received the highest score 8 due to their smooth skin with minimal wrinkles. In infested fruits, the appearance of the fruits is quite poor. The fruits had scars and several surface defects, and the surface of the peel was blotchy. The colour of the fruit in the sample of uninfested fruit was given the highest score of 9 due to uniformity of colour, while the samples of infested fruit were given the lowest score of 2. Sooty mold was present on all fruit and significantly affected the aesthetic value of the fruit. The size of the fruit was also rated better on the uninfested fruit. Fruit flavour was subjective, but a large difference in flavour was observed between uninfested and infested fruit. Uninfested fruits were more aromatic, juicy, and sweeter, while infested fruits had less juice than uninfested fruits and tasted sour. No seeds were found in the uninfested and infested fruits, which is why they received the highest score of 10.

Table 2 shows the results for average weight, height, width (diameter), fruit shape index, and areola diameter. In the uninfested fruits, only 6 fruits had an areola, while in the infested fruits, only one fruit did not have an areola. However, the average number of segments per fruit was the same in both samples (10).

Table 2. *Analysis of variance of pomological properties of uninfested and infested mandarin fruits, Owari'*

FRUIT	**Fruit weight (g)**	**Fruit height (mm)**	**Fruit width - diameter (mm)**	**Fruit shape index**	**Areola diameter (mm)**
Uninfested	$89{,}95 \pm 11{,}08^a$	$53{,}29 \pm 2{,}98^a$	$60{,}15 \pm 4{,}21^a$	$0{,}89 \pm 0{,}05^a$	$16{,}68 \pm 0{,}76^a$
Infested	$58{,}85 \pm 7{,}29^b$	$41{,}62 \pm 2{,}41^b$	$53{,}92 \pm 3{,}03^b$	$0{,}77 \pm 0{,}05^b$	$17{,}97 \pm 2{,}48^a$
Pr > F	$P \leq 0{,}0001$	$P \leq 0{,}0001$	$P \leq 0{,}0013$	$P \leq 0{,}0001$	$P \leq 0{,}1341$

Average values and standard deviations are shown. Different letters added to the average values indicate a statistical difference between the average values

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Uninfested mandarin fruits had an average weight of 89.95 g, while the weight of infested fruits was 58.85 g. According to a study by Radulović et al. (2005), the average weight of the mandarin variety *Unshiu* ranged from 82.5 to 96.3 g, while in a study conducted in Spain, the average weight for the variety Satsuma Owari was 86 g (Gonzales-Silicia, 1951), while in Turkey, the average weight for Satsuma Owari ranged from 83.5 to 126.6 g (Hepaksoy, 2004). The results of the average fruit weight in the conducted study are consistent with the results of the average fruit weight of an uninfested sample, while the average fruit weight of the infested sample is much lower, which is confirmed by a statistically significant difference (P≤0.0001). Uninfested fruits have an average width of 60.15 mm and a height of 53.29 mm, while the average width of infested fruits is 53.92 mm and a height of 41.62 mm. Bakarić (1983) states that the width of mandarinfruit is between 3.5 and 7.0 cm and the height is between 3.0 and 5.0 cm, which is in agreement with the results of uninfested and infested fruits. According to the obtained results, the uninfested fruits belong to the Extra class, as they are well developed, have no defects in appearance and their diameter is within the specified parameters of the Extra class (54-64 mm), while the infested fruits belong to class I in diameter (44-54 mm), but due to the damage of the rind and poorer quality they are classified in the class II (Skendrović Babojelić, 2009). The index of the uninfested fruit shape is 0.89, and of the infested is 0.77. Normally, the ratio between the width and height of the fruit is about 1.3:1, and this is a fairly stable size (Bakarić, 1983). The study conducted in Mexico on *Unshiu* mandarins showed an average fruit index of 0.86 (Ebel et al., 2004), which is in agreement with the results of uninfested fruits, which are larger than infested ones, and a statistically significant difference was found (P ≤0.0001). The areola diameter of an uninfested fruit is 16.68 mm and that of an infested one is 17.97 mm. The areola is located at the tip of the fruit and is usually round, may be convex or raised, is considered more of a varietal characteristic, and its size also depends on the size of the fruit (Tadeo et al., 2020). There was no statistically significant difference in the size of the areola between infested and uninfested fruits, and the areola was imprinted in both samples.

The results of the analysis of physicochemical properties of uninfested and infested fruits of mandarin 'Owari' (soluble dry matter, total acids, citric and malic acid concentration, ash content, total phenolic content, vitamin C, and pH) are shown in *Table 3*.

Table 3. *Analysis of variance for physicochemical properties of uninfested and innfested mandarin fruits, Owari*

FRUIT	Soluble dry matter (°Brix)	Total acids (% citric)	Citric acid (g/L)	Malic acid (g/L)	Ash (g/100g)	Total phenolic content (mg GAE/g)	Vitamin C (mg/100 g)	pH
Uninfested	12.7±0.06[a]	1.23±0.06[a]	8.93±0.12[a]	2.43±0.06[a]	0.39±0.01[a]	64.5±0.64[a]	30.67±1.15[a]	3.49±0.01[b]
Infested	10.2±0.06[b]	0.81±0.01[b]	6.87±0.06[b]	0.93±0.06[b]	0.30±0.01[b]	43.7±0.21[b]	21.66±1.15[b]	3.55±0.03[a]
Pr > F	P ≤0.0001	P ≤0.0002	P ≤0.0001	P ≤0.0001	P ≤0.0004	P ≤0.0001	P ≤0.0007	P ≤0.0474

Average values and standard deviations are shown. Different letters added to the average values indicate a statistical difference between the average values

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It can be seen from *Table 3* that the values of all parameters differ significantly. The amount of soluble dry matter of uninfested fruits is 19.6% higher than that of the infested fruits. The soluble dry matter of the uninfested fruit samples was 12.7 ° Brix, while that of the infested fruit samples was lower at 10.2 ° Brix. Statistically significant differences ($p \leq 0.0001$) between uninfested and infested fruit were found for soluble dry matter content. In research effects of different freezing methods on quality of mandarin, soluble dry matter of control uninfested fruit sample Satsuma mandarin was 12.0 ° Brix (Devseren et al., 2018). In the study of mandarins from the Neretva region, the value of soluble dry matter for the variety 'Owari' was 13.1 ° Brix (Levaj, 2009). The value of soluble dry matter of the uninfested fruit sample in this study agrees with the study of the mentioned author. In 2005 and 2006, a study was conducted on the optimal pre-treatment of fruit and the method of storage while maintaining the quality of the fruit. Only uninfested and undamaged fruits from the Neretva Valley were used in the research. At the beginning of the study, the initial soluble dry matter was 11.29 ° Brix (Skendrović Babojelić et al., 2008; Skendrović Babojelić, 2009), which is consistent with the current study, while in 2006 it was 9.57 ° Brix, which is 15.2% less than in 2005. The same author notes that the amount of soluble dry matter increases with increasing fruit size, i.e. ripening, and changes from year to year under the influence of climatic conditions. The lower amount of soluble dry matter may also be influenced by lower temperatures in plantations during fruit set, due to lower carbohydrate accumulation and a smaller increase in soluble dry matter and a smaller decrease in total acids (McAneney et al., 1995). At physiological maturity, fruits usually contain between 0.70 and 1.20% total acids (titration) expressed as citric acid (Bakarić, 1983). In this study, the total acidity of unaffected fruit was 1.23%, while of affected fruit was 0.81%. According to the data obtained, both samples reached physiological maturity. Skendrović Babojelić (2009) indicates a total acidity content of 1.19% in 2005 and 1.34% in 2006. The reported data of total acidity for two consecutive years are consistent with data from an uninfested sample. In a study by Xu et al. (2008), the total acidity of mandarins was found to range from 0.94-1.87%. The total acidity of an uninfested fruit coincides with the results of the study of the mentioned author, while the infested fruit has a lower acidity than in the study. The values of total acids of the studied samples showed statistically significant differences between samples ($p \leq 0.0001$). In this study, the citric acid of a uninfested fruit was 8.93 g/L, while the value of the infested fruit was slightly lower at 6.87 g/L, which is a statistically significant difference between samples ($p \leq 0.0001$). As for malic acid, 2.43 g/L was found in uninfested fruit, while 0.93 g/L in the infested fruit. Yun et al. (2010) found that citric acid accounts for up to 90% of the total organic acid content during ripening. In the study of basic parameters of *Unshiu* 'Owari' for the value of citric acid was obtained 8.4 g/kg (Özkaya et al., 2019), which agrees with the obtained result of this study of uninfested fruits. The values obtained for organic acids (malic acid, citric acid) showed significant differences between samples ($p \leq 0.0001$). Mandarin fruit is a good source of phenolic compounds. The amount of total phenolics in the uninfested fruits of the studied cultivar 'Owari' was 64.5 mg GAE/g, while in the infested fruits it was 43.7 mg GAE/g. Ye et al. (2011) found concentrations of phenolic compounds ranging from 47.1 to 78.7 mg GAE/g. The values for phenolic compounds for uninfested and infested fruit are consistent with those of the cited author. The ash content of a uninfested sample was 0.39 g per 100 g of fruit, while the ash content of the infested mandarin was 0.30 g per 100 g of fruit. According to the USDA database (2017), mandarins contain 0.38 g of ash per 100 g of fruit. The value determined for the ash of

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the uninfested fruit is consistent with the reported values, while the value for the infested fruit is lower. In the infested fruits studied, the vitamin C content was 22 mg/100 g, while the uninfested fruits contained more vitamin C, 31 mg/100 g. The vitamin C content in mandarin fruit usually ranges from 15 to 50 mg/100 g (Martí et al., 2009; Kelebek and Selli, 2014). The determined values for vitamin C are in agreement with the studies of the mentioned authors. Bakarić (1983) states that higher vitamin C content was found in smaller fruits, but this was not the case here. The determined values of phenolic compounds, ash and vitamin C of the studied samples showed significant differences. The pH of the uninfested fruits was 3.49, while of the infested ones was 3.55. Levaj (2009) shows that cultivar 'Owari' from the Neretva region, gives a pH of 3.62 similar to Andersen et al. (2020) who show that same Cultivar gives a pH 3,70. The values of the studied samples are lower than in the studies conducted so far on the variety 'Owari', and the differences between uninfested and infested fruits are significant ($p \leq 0.0001$), which correlates with the values of total acidity. The differences between the above studied traits are most likely the result of the effect of many factors, especially external factors. In this study, this refers to the insect *A. spiniferus*, which directly and indirectly affects the chemical composition and quality of the fruits.

Table 4 shows the results of the analysis of mineral composition (nitrogen, potassium and phosphorus) in uninfested and infested mandarin leaves 'Owari'.

Table 4. *Analysis of the variance of mineral composition of uninfested and infested mandarin leaves, Owari's harvested in October*

LEAF	**Total nitrogen (N - %)**	**Total phosphorus (P - %)**	**Total potassium (K - %)**
Uninfested	$2,58^{a} \pm 0,05$	$0,20^{a} \pm 0,01$	$1,56^{a} \pm 0,01$
Infested	$2,24^{b} \pm 0,01$	$0,16^{a} \pm 0,03$	$1,29^{b} \pm 0,02$
Pr > F	P ≤0.0005	P ≤0.1583	P ≤0.0004

Average values and standard deviations are shown. Different letters added to the average values indicate a statistical difference between the average values

An uninfested mandarin leaf contains 2.58% nitrogen, 0.20% phosphorus, and 1.56% potassium. The optimal range for N in citrus leaves is 2.5 - 2.7%, for P 0.12% - 0.16%, and for K 0.90 - 1.09% (Lovatt, 2014). According to the guide for the analysis of citrus leaves (Lovatt, 2014), the analysed uninfested mandarinleaf contains the optimum proportion of nitrogen and a high proportion of phosphorus and potassium. The higher phosphorus content reflects the higher sugar content of the uninfested fruit, while the higher potassium content is due to the texture, weight, and quality of the fruit, as evidenced by previous analyses (*Table 3*). The analysis of infested mandarin leaves showed concentrations of 2.24% nitrogen, 0.16% phosphorus and 1.29% potassium. The determined values for nitrogen and potassium concentrations in the studied samples showed significant differences between the uninfested and infested leaf ($p \leq 0.0001$). The analysis of the infested leaf showed a deficiency in nitrogen concentration (2.24%), which is consistent with the theory that nitrogen content is reduced in leaves infested with honeydew and soot fungi (Šimala et al., 2013). Indeed, in the studied plantation, the trees and leaves are less vigorous, the young leaves are pale yellow, and the germination of flower buds and fruits is reduced, which could be due to reduced nitrogen concentration.

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In addition to the lack of nitrogen concentration in the leaf, the infested leaf studied contains an optimal concentration of phosphorus and a high concentration of potassium. Dowell (1983) also reported lower nitrogen concentrations in whitefly-infested leaves and loss of other nutrients important for fruit ripening in his study. Due to the release of honeydew on leaves and the establishment of the sooty mold, gas exchange is disrupted through the leaf stomata, i.e., respiration and transpiration are disrupted, and leaf temperature increases, leading to leaf death and complete defoliation (Bryne et al., 1990). Brink and Hewitt (1992) note that heavily infested leaves lack photosynthetic activity and that removal of the fungal layer from the leaves leads to the resumption of photosynthesis. Chomnunti et al. (2014) strongly suggest that plantation management is important because pests develop faster in high humidity and high temperatures, leading to faster production of honeydew and faster growth of soot fungi. The abundant honeydew covered with sooty mold on leaves and shoots results in poorer flowering and fruit set, new shoots are in poorer condition, stunted, and the combination of all these symptoms results in poorer fruit texture and quality, i.e., the developed fruits are smaller and wrinkled (Ba-Angood, 1977). Another, but no less important, consequence of the infestation is reduced resistance to cold (English and Turnipseed, 1940), which is of great importance for our breeding area.

Conclusion

In this study, the influence of the quarantine pest *Aleurocanthus spiniferus* on the pomological and physicochemical characteristics of fruits and mineral composition of mandarin leaves (*Citrus Unshiu* Marc.'Owari') was presented. The pest infestation significantly affected the pomological characteristics of the fruits, and statistically significant differences were found in the values of weight, height and width between healthy and contaminated fruits. Fruit quality analysis also revealed poorer quality of fruits affected with sooty mold. Based on the data obtained, statistically significant differences were found in soluble dry matter, total acidity, ash, total phenols and vitamin C. Fruits infested with sooty mold also had a significantly poorer appearance, which reduced their aesthetic and market value. The results of mineral composition of leaves showed a statistically significant difference in nitrogen and potassium concentration in contaminated and healthy fruits, while no significant statistical difference was found in phosphorus concentration. Future research will provide a more comprehensive analysis of the harmfulness of this quarantine pest to the productivity and vigour of other citrus fruits. In addition, physicochemical analysis of infested fruit will be conducted. The analyses will be carried out because of the changes in chemical composition and their impact on the quality and, consequently, on the commercial value of the fruit itself, leading to a reduction in economic viability. Therefore, it is extremely important that commercial growers and owners of mandarin and other citrus trees thoroughly inspect the plants for the presence of this quarantine pest to control it in time to prevent its impact on citrus fruits.

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