

**RESULTS OF A TWO YEAR SURVEY (2015-2016) OF QUARANTINE
WHITEFLY SPECIES FROM GENUS *Aleurocanthus* Quaintance & Baker
1914 ON CITRUS IN CROATIA**

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ABSTRACT

Citrus production in Croatia is of great importance in agriculture and national economy. The most important production areas are located in central and southern Dalmatia, with mandarin production in the Neretva river valley being of greatest importance. Whiteflies (Hemiptera: Aleyrodidae), in particular non-European invasive species have become increasingly important pests of citrus in the Mediterranean basin, including Croatia. The greatest phytosanitary threat to the production of citrus in Croatia and other Mediterranean countries from the insect family Aleyrodidae represent quarantine species *Aleurocanthus spiniferus* Quaintance, 1903 and *Aleurocanthus woglumi* Ashby, 1915. High risk of introduction of these quarantine species was one of the main reasons for their inclusion on the list of priorities for survey during 2015 and 2016, which was implemented as a part of the national surveillance programme of citrus harmful organisms listed in Annex IAI and IIAI of Directive 2000/29/EC. The main objective of the two year survey in citrus plantations was to determine the possible presence and current status of quarantine whitefly species from genus *Aleurocanthus* in four coastal counties (Zadar, Šibenik-Knin, Split-Dalmatia and Dubrovnik-Neretva), particularly after the first finding and successful eradication of species *A. spiniferus* in Croatia in 2012. Presence of *A. spiniferus* and *A. woglumi* was investigated in citrus orchards, gardens and public areas on a total of 50 localities on the coast and on the islands. Visual inspections with sampling of leaves infested with whitefly larvae were combined with trapping of adults using yellow sticky traps. The whiteflies collected in leaves' samples were identified to the species level on the basis of morphological characters of puparium and pupal case, using classical identification method according to the relevant morphological keys. *Aleurocanthus* species were not recorded in Croatia in 2015 and 2016. The most common species in samples of collected leaves was *Dialeurodes citri* Ashmead, 1885, a very important pest on all *Citrus* species throughout the citrus growing area in Croatia. Another very commonly identified whitefly species was

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Aleurothrixus floccosus Maskell, 1896. Japanese bayberry whitefly *Parabemisia myricae* (Kuwana, 1927) was found for the first time in Croatia in 2015 in lemon plantation, in Lumbarda on the island of Korčula.

Key words: *Aleurocanthus*, Aleyrodidae, citrus, Croatia, survey

1 INTRODUCTION

In Croatia, citrus are commercially grown in the southern coastal region of Dalmatia, including the islands. This region lies between 42° and 44° of the northern latitudes and represents probably the northernmost commercial citrus growing area in the world. Citrus production is mostly limited to the cold tolerant Satsuma mandarin plantation in the Neretva river valley, with annual production of about 40 000 tons of fruits on around 1500 hectares, while lemon and sweet orange are grown in warmer micro-locations (Škorić et al., 2002). The whitefly genus *Aleurocanthus* currently comprises around 80 described species worldwide (Martin & Mound 2007; Evans 2008). It is well represented in the Oriental region, with about 50 described species. Species *A. spiniferus* and *A. woglumi* are serious citrus pests of Asian origin, regulated (as *Aleurocanthus* species) by EU Annex IIA1 Council Directive 2000/29/EC (Anonymous, 2000), which prevents the introduction and spreading of all the species within the genus in all member states. In Europe, *A. spiniferus* has so far been detected in Italy in 2008 (Porcelli, 2008), in Croatia in 2012 (Šimala & Masten Milek, 2013) and in Montenegro in 2013 (Radonjić & al., 2014). For now, it has a status of eradicated species in Croatia. *A. woglumi* has so far not been introduced in the EPPO area. Both species are extremely polyphagous, with strong preference to citrus as host plants. They feed on plant sap and excrete copious amounts of sticky honeydew, which coats the leaves, branches and fruit surfaces. Black sooty mould develops on honeydew, reducing respiration and photosynthesis. As a result, contaminated foliage may drop, young leaf growth is damaged, fruit set may be reduced, and already formed fruits are unmarketable. High risk of a reintroduction of species *A. spiniferus* and first introduction of *A. woglumi* into Croatia, especially with imported ornamental citrus cultivars, but also with other host plants, and their potential phytosanitary threat to the mandarin production in the Neretva river valley, were the reasons for starting a survey in 2015.

2 MATERIALS AND METHODS

A survey of quarantine whitefly species *A. spiniferus* and *A. woglumi* was conducted in 2015 and 2016 in citrus plantations in Zadar, Šibenik-Knin, Split-Dalmatia and Dubrovnik-Neretva counties. Citrus plantations were inspected rarely once, mostly twice and in 2016 even four times during the vegetation. Possible presence of dark adult whiteflies from genus *Aleurocanthus* was established by hanging of 5 yellow sticky traps per plantation, which represented one sample from a certain location. Yellow sticky traps were afterwards analysed in the laboratory under stereomicroscope Olympus SZX 7. Whitefly nymphal and pupal instars were collected during visual

inspections of the leaves' under-surface. Ten to twenty trees in each citrus orchard were inspected, with the help of a magnifying lens of 10 times magnification. Samples of 5-10 infested leaves were collected and stored dry in envelopes until whitefly laboratory examination (Martin, 1987; 1999). A series of several puparia and/or empty pupal cases from each collected sample were slide mounted in Canada balsam according to modified Watson & Chandler (1999) method, examined using an Olympus BX 51 high power microscope (magnification x100-400) and identified on the basis of morphological characteristics using the descriptions and keys provided by Martin & *al.*, 2000 and Uygun *et al.*, 2010. Identity of the whitefly species *Parabermisia myricae* (Kuwana 1927) was confirmed at KGZS, Nova Gorica, Slovenia. Slide-mounted specimens were deposited in the collection of the laboratory for zoology of the Institute for Plant Protection - Croatian Centre for Agriculture, Food and Rural Affairs.

3 RESULTS AND DISCUSSION

During 2015 and 2016, a survey of quarantine whitefly species *A. spiniferus* and *A. woglumi* was conducted in mandarine, lemon and orange plantations on a total of 50 localities (30 in Dubrovnik-Neretva county, 18 in Split-Dalmatia and 1 locality in Zadar, and Šibenik-Knin counties, respectively) on the coast and on the islands (Figure 1).



Figure 1: Localities where survey of *Aleurocanthus* spp. was conducted in 2015 and 2016.

Possible presence of both whitefly species was checked by visual inspections of host plant leaves on the infestation of larvae and by hanging of yellow sticky boards on citrus plants for the purpose of catching adult whiteflies. Altogether 36 samples of yellow sticky boards were collected from citrus plantations for laboratory analysis during a two year survey (Figure 2). Adult whiteflies of species *A. spiniferus* and *A. woglumi* were not determined in any of the analysed samples. Since the taxonomy of Aleyrodidae is mainly based on the morphology of slide-mounted puparia or empty pupal cases, presence of dark whitefly adults on yellow sticky boards would just be

the sign of *Aleurocanthus* spp. infestation in specific citrus plantation, but not scientific evidence.

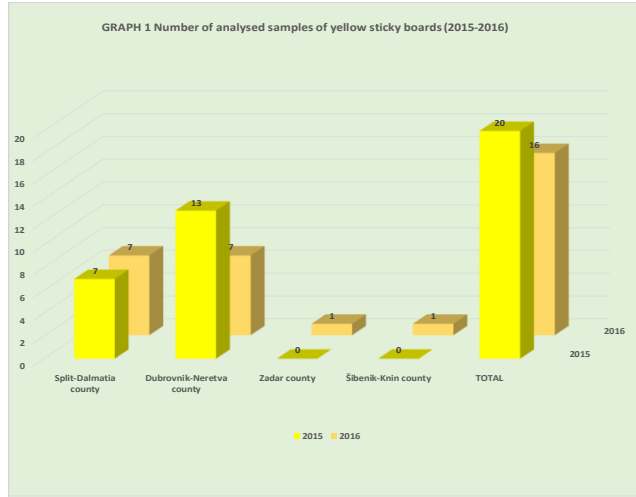


Figure 2: Number of analysed samples of yellow sticky boards (2015-2016).

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Whiteflies from a total of 60 leaf samples collected during 2015 and 2016 were identified to the species level, on the basis of morphological characteristics of puparia and/or empty pupal cases (Figure 3). All collected leaf samples were negative for the presence of quarantine whitefly species.

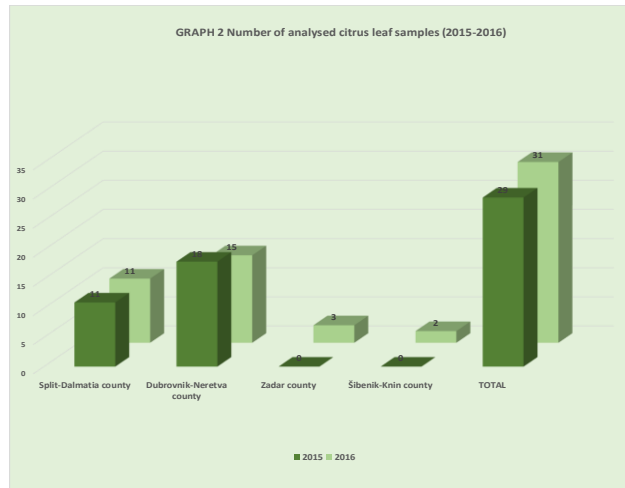


Figure 3: Number of analysed citrus leaf samples (2015-2016).

According to Uygun *et al.* (2010), six species of whiteflies are major citrus pests in the Mediterranean area. Survey in citrus plantations in Croatia has resulted in finding of three of them (Figure 4).

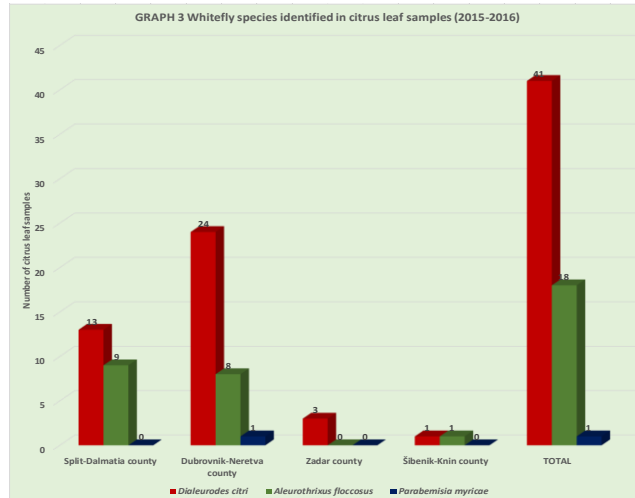


Figure 4: Whitefly species identified in citrus leaf samples (2015-2016).

D. citri was the most common whitefly species in samples of collected leaves. It invaded citrus plantations in Croatia in 1997 (Bakarić, 1983) and soon after it became the most important whitefly pest on all *Citrus* species throughout the citrus growing area. Citrus whitefly is still dominant whitefly species on citrus in Croatia. Another very commonly identified whitefly species was *A. floccosus*. Woolly whitefly was recorded in Croatia in 2007 on citrus in limited area of the Croatian Middle Adriatic region (Žanić, 2007). However, according to the results of the survey, this species expanded its distribution to the majority of citrus production areas in central and southern Dalmatia. The third recorded whitefly species was *P. myricae*. This East Palearctic whitefly species native to Japan, was first found in Croatia in September 2015 on lemon trees in Lumbarda (N 42°55'20.9" E 17°10'16.8") on the island of Korčula (red mark on Figure 1). *P. myricae* is a polyphagous pest, widely distributed across the Mediterranean Basin, where avocado and citrus are major hosts. It represents a potential phytosanitary risk for citrus production in Croatia. In the field, the puparium of *P. myricae* is broadly suboval and flatten, white to yellowish, with a translucent wax fringe that borders the body. Slide-mounted pupal case has an outer submargin with a row of 14 pairs, including caudal pair of evenly spaced and even-sized setae. Vasiform orifice is triangular to elongate-cordate with operculum occupying its basal half and elongated lingula with characteristic two blunt laterobasal lobes and two long setae.

4 CONCLUSIONS

A two year survey of quarantine whitefly species *A. spiniferus* and *A. woglumi* conducted in citrus plantations in 2015 and 2016 on a total of 50 localities in four coastal counties of Croatia has not resulted in their finding. Altogether 60 leaf samples and 36 samples of yellow sticky boards were collected from citrus plantations for laboratory analysis and whitefly species identification. Species *A. spiniferus* and *A. woglumi* were not determined in any of the analysed samples. Species *D. citri*, *A. floccosus* and *P. myricae* were identified in collected citrus leaf samples. *P. myricae* was found for the first time in Croatia.

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