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DIFFERENTIAL SUSCEPTIBILITY OF OLIVE VARIETIES TO OLIVE KNOT DISEASE IN ISTRIA

RAZLIČITA OSJETLJIVOST SORATA MASLINE NA RAK MASLINE U ISTRI

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ABSTRACT

We performed a survey on susceptibility of two olive varieties 'Leccino' and 'Picholine' to olive knot disease in the area of Poreč in the years 2008 and 2009. The incidence of disease was determined by visual inspection of disease symptoms on olives according to recommendations of International Olive Council (IOC). The meteorological data were collected during 2007 and 2008. The damage caused by the disease was evaluated and the area where symptoms appeared was quantified. A rather high frequency of olive knot symptom was determined at all locations investigated. The new infection incidence was lower in 2009 for both varieties probably due to the winter copper treatment conducted. The variety 'Picholine' showed higher incidence of disease.
Keywords: dissemination, Istria, meteorological data, olive variety, *Pseudomonas savastanoi*

SAŽETAK

Proveli smo istraživanje osjetljivosti dviju sorti masline 'Leccino' i 'Picholine' na rak masline na području Poreča 2008. i 2009. godine. Učestalost je bolesti određena vizualnim pregledom prema skali prepuručenoj od Međunarodnog vijeća za maslinu (IOC). Meteorološki su podaci prikupljeni tijekom 2007. i 2008. godine. Procijenjena je šteta i kvantificirano je područje zaraženosti u kojem su uočeni simptomi. Utvrđena je vrlo visoka učestalost simptoma raka na svim ispitivanim lokacijama. Brojnost novih zaraza je bila manja u 2009. godini kod obje sorte vjerojatno zbog prethodno provedenog zimskog tretiranja bakrom. Sorta 'Picholine' je ukupno gledano imala veću učestalost pojave bolesti.
Ključne riječi: Istra, meteorološki podaci, *Pseudomonas savastanoi*, rak masline, sorta masline

DETAILED ABSTRACT

Naše je istraživanje provedeno u jesen 2008. i 2009. godine u masliniku u okolici Poreča na dvjema sortama masline: talijanskoj sorti 'Leccino' i francuskoj sorti 'Picholine'. Meteorološki su podaci prikupljeni u 2007. i 2008. godini u meteorološkoj stanici udaljenoj oko 5 km od proučavanog maslinika. Ukupno je na pet istraživačkih lokacija u 2008. godini ispitano 50 stabala, a u 2009. godini 110 stabala. Učestalost bolesti i stupanj zaraze procijenjeni su na svakoj lokaciji na temelju vizualnog pregleda grana stabala pritom koristeći skalu kategorija zaraze Međunarodnog vijeća za maslinu (IOC). Utvrđena je visoka učestalost simptoma raka masline na svim lokacijama u 2008. i 2009. godini. Sve kategorije kojima se izražava jačina zaraze su bile prisutne u obje godine istraživanja, osim kategorije oznake 1 (0% zaraze, bez znakova bolesti). Sorta 'Picholine' je pokazala najviši stupanj zaraze. Prosječna je učestalost zaraze u 2008. godini sorte 'Leccino' iznosila 27,5%, a sorte 'Picholine' 73%, dok je u 2009. prosječna zaraza sorte 'Leccino' bila 11,87%, a sorte 'Picholine' 54,75%. Optimalni vremenski uvjeti za razvoj bolesti (relativna vlaga iznad 80%) su prevladavali od siječnja do ožujka 2007. godine kao i u siječnju 2008. g., dok su srednje dnevne temperature iznad 18°C bile prisutne od svibnja do rujna 2007. i 2008. godine.

Pretpostavljamo da su glavni uzroci širenja bolesti bile agrotehničke mjere, osobito mehanizirana berba. Bez obzira na sve, prosječna je zaraženost sorte 'Leccino' u 2009. godini bila 2,32 puta manja s obzirom na prethodnu godinu. Kod sorte 'Picholine' je ova razlika u prosječnoj zaraženosti između godina zabilježena kao smanjenje od 1,34 puta. Pretpostavlja se da je uzrok tom poboljšanju stanja tj. smanjenju frekvencije zaražavanja vjerojatno provedeno zimsko prskanje bakrom.

INTRODUCTION

Bacterium that is causative agent of olive knot symptom was isolated and described by Luigi Savastano and named as *Bacillus oleae tubercolosis*. Later, in 1908, it was called *Bacterium savastanoi* by Smith. The current name is *Pseudomonas savastanoi* pv. *savastanoi* and is given in 1992 by Gardan *et al*. according to [14].

Olive knot is probably the first plant disease to be clearly described in Antiquity. In the years 370-286 BC the philosopher Theophrastus described it as nail, fungus or little bowl. Pathogen is a short rod aerobic gram-negative bacterium belonging to the order *Pseudomonadales*, family *Pseudomonadaceae*; having 1-4 polar flagellae with dimensions of 0.4-0.8 μm x 1.2-2.3 μm [2]. The bacterium lives as an epiphyte on healthy leaves and when weather conditions are optimal (22-30°C and over 80% of relative humidity) it may become a source of infection [15, 2, 8]. In the arid summer and cold winter the bacteria enter deeply into the inner parts of tumours and in that way to next year brings the 90% of its infection potential [17]. It is the causal agent of olive knot disease, a bacterial disease that affects olive trees mainly in Mediterranean countries, where climatic conditions often favour disease spread [13]. The minimal temperatures of 18-22°C are essential for the onset of the disease [7]. Trees are very susceptible in the intensive meristematic activity which is involved in the process of pathogenesis [6]. Tissues can be infected through leaf scars formed when leaves fall, wounds caused by pruning or harvesting and fissures on stem and twigs. Leaf scars are the most common sites of infection and they are most susceptible to infection during the first 2 days after the leaf falls and remain

vulnerable for another 7 days. Excessive leaf fall coupled with rain in spring (March through June) may result in more severe olive knot disease [16].

Wounds can also be caused by meteorological phenomena (hail and frost) and insect miners, as well as injures caused by harvest and pruning practices. After pruning outgrowths can be seen at the pruning site between wood and cambium [17]. Depending on the climatic conditions, the incubation period may last from one month (end of spring and during summer) till three months (in winter) [3, 17]. The dissemination of the inoculum occurs prior to rain and wind, especially in the period spring-autumn when the bacterial population is elevated (up to 10^4 bacteria/cm^2 on twigs or leaves) unlike winter period when the population is lower [8, 6]. From there it can colonize other natural or artificial openings on the tree or stem and leaves as an epiphytic bacterium [14]. It moves also across the vessels into other plant organs and there originates secondary knots. Seed transmission of the pathogen has never been documented, while the olive fruit fly can contain the bacterium [14]. Symptoms are characterized by tumourous outgrowths of plant cell tissue on different parts of infected plants, mainly shoots and branches. The galls are initially small protuberances or swellings. They are growing quickly and turn into smooth, soft, spherical “knots” (3-5 mm in diameter) that are covered with greenish bark. With age, the galls grow bigger and became hard, brown to black and corrugated, with a size ranging from a few millimetres to several centimetres in diameter [9]. The development of these galls is dependent on bacterial production of the phytohormone indole-3-acetic acid (IAA) and cytokinins [13].

Olive knot disease is considered an important problem for olive crops because of its effect on vegetative growth, olive yield and even possibly on olive oil quality through inferior organoleptic characteristics such as an unpleasant smell and a bitter, rancid taste [2, 8, 1].

In other fruit trees *Pseudomonas* spp. is usually considered a minor pathogen causing only qualitative damage. In fact damage can be high when associated with frost damage. Blossom blast of fruits can cause high economic loses while twigs and bark cankers weaken general fitness of trees increasing their susceptibility [4].

Olive knot can cause the decline of branch and shoots, death of small branches and twigs and also lower yields. Trees moderately infected (0.5-1 knot per 30 cm length of branch) give lower yield of about 28% beside the ones with low infection (0.1-0.3 knot per branch) [6].

Some studies have found that different olive genotypes have different degree of susceptibility to bacterium *P. savastanoi*. According to [18] the variety ‘Leccino’ is quite resistant to olive knot. Like that, the use of tolerant or resistant varieties is considered one of the most appropriate methods of control at this moment, because the peak of the disease depends on susceptibility of the olive variety [2]. Although there isn’t resistant variety at all, a discrete number of them are considered tolerant to the olive knot [6]. According to that, the varieties ‘Frantoio’ and ‘Pendolino’ resulted highly reactive, while the varieties ‘Ascolana tenera’, ‘Leccino’ and ‘Picholine’ gave an intermediate response. There isn’t note of infections by olive knot on the old autochthonous varieties in Poreč area [17]. According to [7] the variety ‘Bianchera’ showed 0% of infection, while the variety ‘Rosulja’ only 16.67%, which is of great importance for the resistance of Istrian olive varieties consideration.

However, the behaviour of the same variety toward olive knot can differ in a distinguishable way depending on different pedoclimatic parameters in various agroecosystems [6].

Olive knot spreads systemically through olive tissue along the xylem vessels, and there is currently a risk that nurseries may grow and trade olive plantlets that harbour the olive knot pathogen [9]. Actually, *P. syringae* subsp. *savastanoi* is included in the list of transmissible agents of olive diseases, and its absence in propagating material is advisable for the certification of olive mother plants covered by the scheme for the production of healthy plants for planting [12, 8]. Like this, in the Croatian Regulation [11] olive knot is also considered, beside others, as harmful organism that affect the quality and utility of the propagating material and olive grafted plants.

MATERIALS AND METHODS

Our investigation was carried out during autumn in years 2008 and 2009 in surroundings of Poreč (northwest Istria, Croatia) in the olive grove planted by two olive varieties: Italian variety 'Leccino' (region of origin Tuscany) and French variety 'Picholine' (region of origin Provanse). The pruning at the location was manual, while the harvest was performed both, manually and mechanically. The meteorological data were collected during 2007 and 2008 at the meteorological station Poreč at about 5 km of distance from the researched orchard (Fig. 1-3).

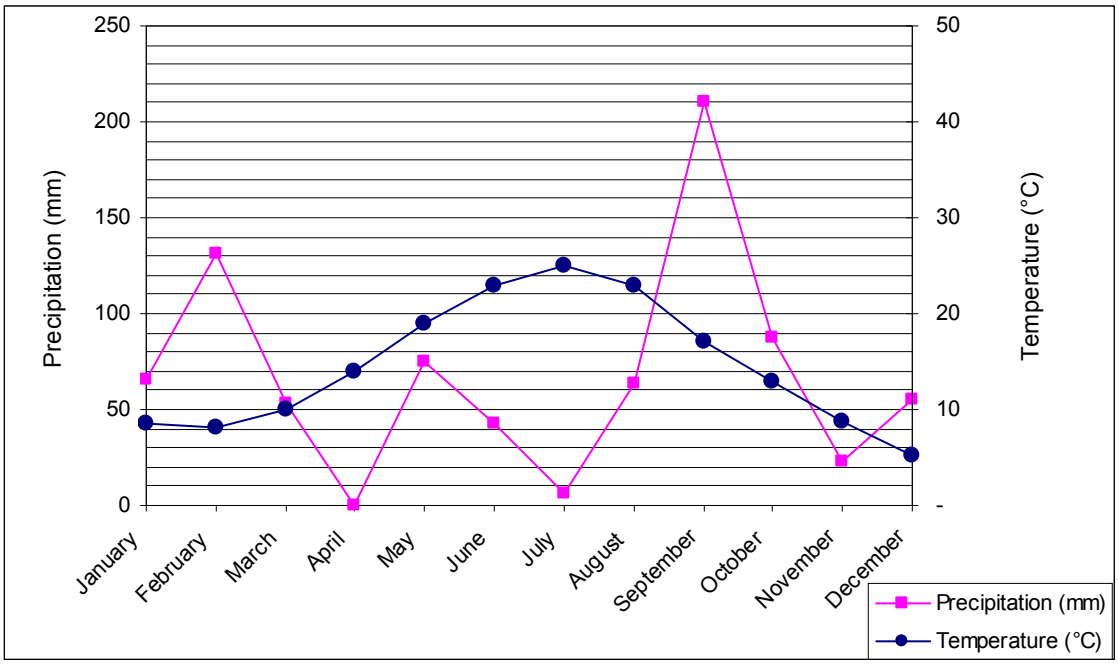

Figure 1. Meteorological data (precipitation and temperature) for location Poreč (averages for year 2007).

Slika 1. Meteorološki podaci (padaline i temperatura) za lokaciju Poreč (prosjeci za 2007. godinu).

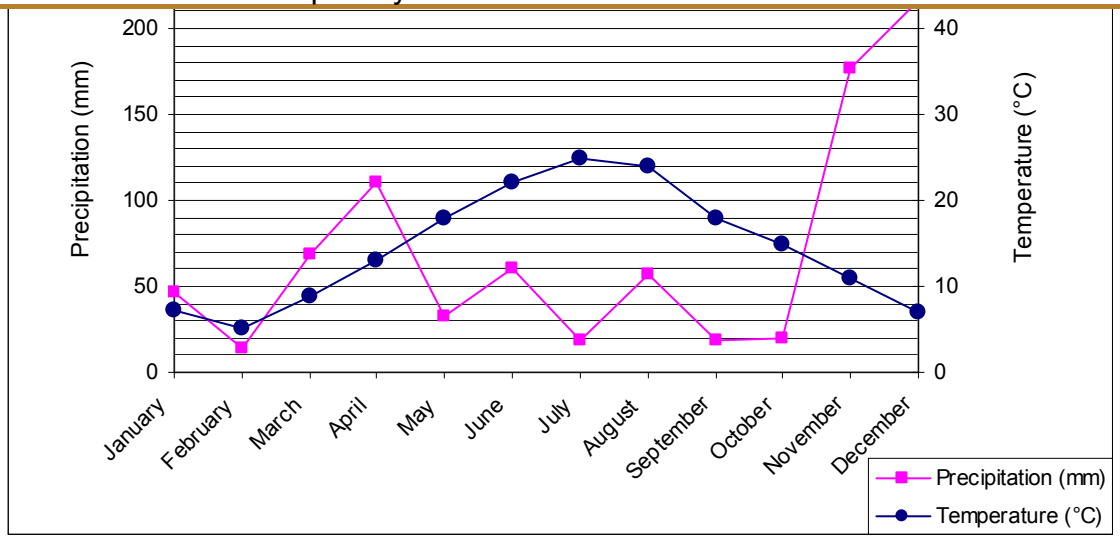

Figure 2. Meteorological data (precipitation and temperature) for location Poreč (averages for year 2008).
Slika 2. Meteorološki podaci (padaline i temperatura) za lokaciju Poreč (prosjeci za 2008. godinu).

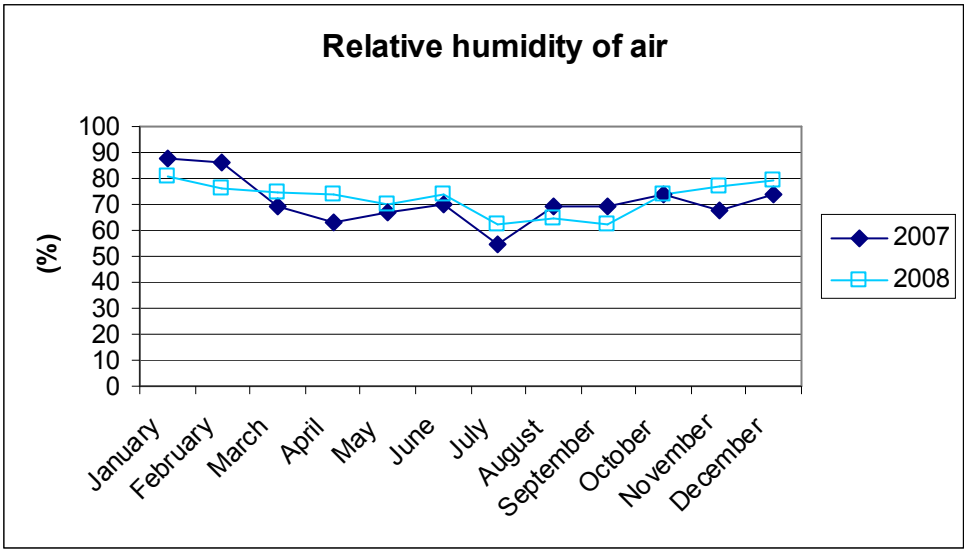

Figure 3. Relative air humidity (%) for location Poreč for years 2007 and 2008.
Slika 3. Relativna vlažnost zraka (%) za lokaciju Poreč za 2007. i 2008. godinu.

In 2008 five separate research locations of about 900 m^2 each were chosen, where 10 trees per site were examined (50 trees or 4500 m^2 in total). In 2009 we performed additional survey on the location 4 ('Picholine') and location 5 ('Leccino') where 40 trees in addition were chosen for further investigations. In 2008 there were analyzed 50 trees in total, while in 2009 there were 110 trees.
Detail data (varieties, age, etc.) about different research locations are presented in Table 1.

Table 1. Data about the researched localities in the orchard near town of Poreč where olive knot disease occurrence was monitored during years 2008 and 2009.
Tablica 1. Podaci o lokacijama u masliniku pored grada Poreča gdje je praćena pojava raka masline u 2008. i 2009. godini.

Location	Altitude (m)	Crop density (m)	Variety	Irrigation	Plant age (years)	No° of trees monitored (2008/2009)
1	16	6x6	Leccino	no	20-30	10/10
2	5	6x6	Leccino	no	20-30	10/10
3	13	5x5	Leccino	no	20-30	10/10
4	16	4x5	Picholine	yes	10-15	10/40
5	19	4x5	Leccino	yes	20-30	10/40

The medium annual temperature in 2007 was 14.5°C, while the total annual precipitation was 813.5 mm and the average relative air humidity was 71%. In 2008 the medium annual temperature was 14.4°C, the total annual precipitation was 840 mm, and the average relative air humidity was 71.81% (Fig. 1-3).

The presence of the disease was estimated on the basis of the quantity of tubercles present on branches. Frequency and degree of contamination were defined by visual examination of upper parts of olive trees, using the scale based on six categories according to International Olive Council (IOC) [10]. The average of infection incidence on each location was determined and statistically interpreted using one-way analysis of variance (ANOVA, Duncan's test).

RESULTS

Based on the research that was conducted in November 2008 and 2009, we found olive knot symptom, presumably caused by bacterium *Pseudomonas savastanoi* on olive trees at all 5 investigated locations in both years with different degrees of infection according to scale of IOC based on six categories (Fig. 4).

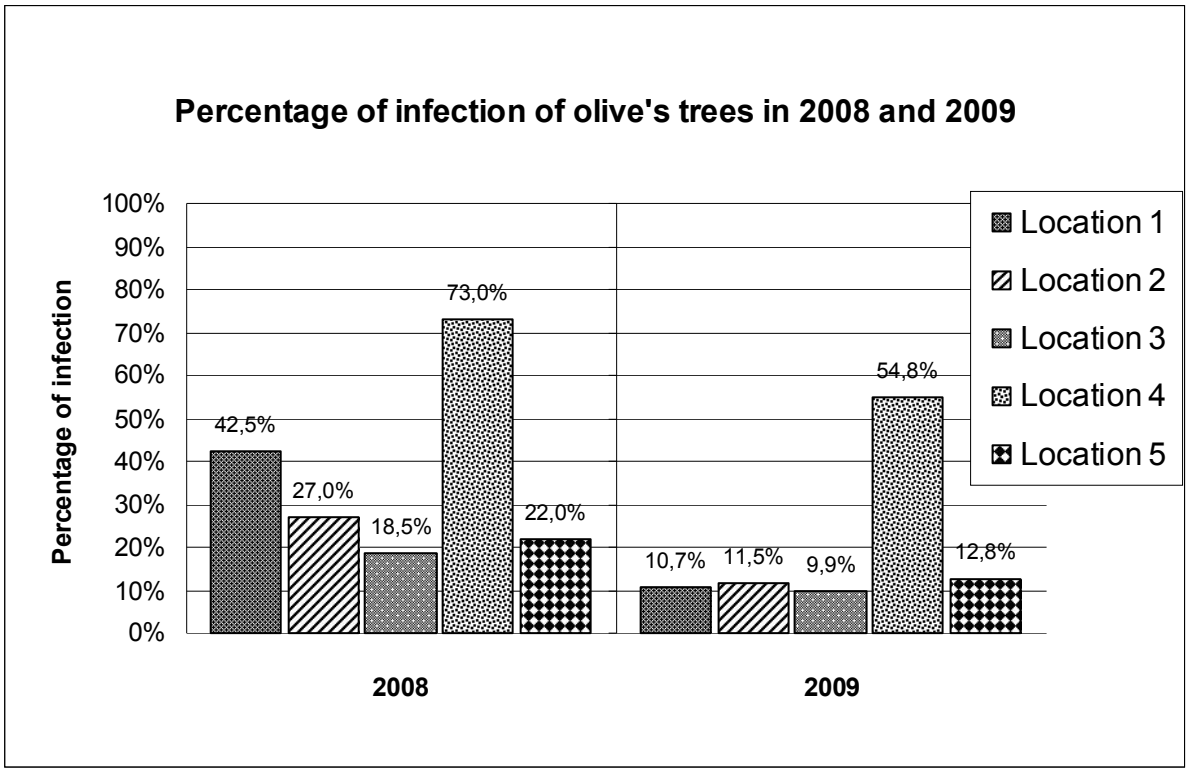

Figure 4. Percentage of olive knot symptom appearance on different locations assayed.

The incidence of symptom appearance of 0% correspond to the category of 1 (nil, no symptoms observed); 1-20% correspond to category 2 (very low); 20-40% correspond to 3 (low); 40-60% correspond to 4 (medium); 60-80% correspond to 5 (high) and 80-100% correspond to category 6 (very high).

Slika 4. Postotak pojave simptoma raka (šuge) masline na različitim istraživanim lokacijama.

Pojava simptoma šuge masline od 0% odgovara kategoriji 1 (nula, bez znakova bolesti); 1-20% odgovara kategoriji 2 (vrlo mala zaraženost); 20-40% odgovara kategoriji 3 (mala); 40-60% odgovara kategoriji 4 (srednja); 60-80% odgovara kategoriji 5 (velika) i 80-100% odgovara kategoriji 6 (vrlo velika zaraženost).

As one can see, the strongest infection in both years was found on location 4. Our results showed also a different situation among two cultivars for both years. The frequency of infection in the year 2008 was in range from 5 to 90% [5], while in the year 2009 it was in range from 3 to 90% (data not shown). In 2008 the minimal percentage of infection (5%, very low infection) was found at the researched location 2, while the greatest percentage (infection of 90%, very high infection rate) was documented on location 4.

In 2009 the minimal percentage of new infection (3%, very low) was present on locations 2 and 3 while the highest percentage of new infections (90%, very high) was found on location 4 twice. All of six IOC's categories [10], except the category 1 (0%), were found in both years. The 'Picholine' variety had higher percentage of infection (location 4) than 'Leccino' variety (locations 1, 2, 3 and 5) in both years. Analogous results were earlier mentioned by other authors, e.g. cultivar 'Leccino' had infection rate of 30% unlike 'Picholine' (70.83%) [7]. Among other varieties, 'Leccino' was found as quite resistant [1].

In the year 2008 the percentage of infection of 'Picholine' was on average 73% (high infection), while in the year 2009 it was 54.75% (medium). The mean percentage for 'Leccino' in 2008 was 27.5% (low) while in 2009 it was 11.87% (very low).

The optimal weather conditions for the onset of the disease (relative humidity over 80%) was present from January till March in 2007 and in January of 2008, while for both years the temperatures of over 18°C was measured from May till September (Fig. 1-3).

In 2008 statistically significant differences ($p<0.05$) were found between localities for variety 'Leccino'. The location 1 showed significant differences compared to other locations checked. In 2009 there weren't statistical differences between localities nor within localities, but there were statistical differences of 5% between years 2008 and 2009.

When speaking about variety 'Picholine', in 2009 there weren't statistical differences within locality found, but there were significant differences of 5% present between two years. For both years there were significant differences between 'Leccino' and 'Picholine'. There were no significant differences found between altitude (m) for 'Leccino' in the years 2008 and 2009.

Figure 5. Injuries on olive tree caused by mechanical harvesting.
Slika 5. Oštećenja na deblu masline nastala u mehaniziranoj berbi.

Figure 6. Development of olive knot disease symptoms after pruning.
Slika 6. Razvoj simptoma bolesti šuge masline nakon rezidbe.

Based on climatic conditions and agrotechnical measures that have prevailed in 2007 and 2008, especially the way how the harvest and the pruning were implemented (Fig. 5 and 6), one can speculate that spread of olive knot disease were severely impacted.

DISCUSSION

From results obtained through our survey one can conclude that a rather high frequency of olive knot symptom was determined at all five investigated locations near town of Poreč in 2008 and 2009. The intensity of infection rate based on visual evaluation according to scale of IOC ranged from very low (category 2) to very high (category 6).

In the climatic conditions of Poreč, olive knot symptoms occur especially on susceptible olive varieties. The selection of varieties and locations, as well as agrotechnical measures and meteorological conditions can affect the abundance of olive knot symptom. In that way we suppose that the main cause of its spreading in surveyed orchard were the agrotechnical measures, especially the mechanical harvesting (Fig. 5).

In consideration of all that was mentioned, it is obvious that the onset of the disease was getting milder with the passage of time. The frequency of new infection in cultivar 'Leccino' in 2009 was nearly 2.32 times lower in respect to 2008 while for 'Picholine' it was 1.34 times lower than in 2008. We suppose that the reason for that improvement is the copper treatment conducted during winter period.

This survey was first effort in monitoring the present status of the olive knot disease in the northwest region of Istrian peninsula. Next steps should be precise determination of causative agent of the knot symptoms, presumably bacterium *Pseudomonas savastanoi*. In the field, in order to reduce the damage, it is necessary to monitor regularly the emergence and intensity of the disease and to implement all available preventive measures of control to reduce its spreading (e.g. the use of non-susceptible varieties on well-drained soils, the application of copper compounds - at leaf fall, after pruning, frost, hail and mechanical harvesting as well as the usage of a wound protecting paste application on cut surfaces after pruning.

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