Comparative morphological analysis of apple blister mite, *Eriophyes mali* Nal., a new pest in Serbia

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SUMMARY

The apple blister mite, *Eriophyes mali* Nalepa, 1926 (Acari: Prostigmata: Eriophyoidea), has been recently found in Serbia as a new pest of apple. The history of its research, the results of a morphological analysis and degree of infestation are presented. A comparison of the main morphological features of mites from different populations of remote geographical origin has shown that the apple blister mite from Serbia is most similar to another European population (Bulgarian [or Austrian?]) while it differs from *E. mali* originating from the USA and New Zealand. The percentage of infestation varied from 1.6% to 87.6%, with an average of 22.4%.

Keywords: Eriophyes mali; Apple; Serbia

INTRODUCTION

Eriophyoid mites (Acari: Prostigmata: Eriophyoidea) are microscopic obligate plant parasites which inhabit all plant parts except roots (Oldfield, 1996, Westphal & Manson, 1996). Many species are considered economically important, causing certain damage to their specific host plants. Eriophyoid mites generally rely on passive dispersal by wind, phoretically by various animals, and through human activities that enable their fast and wide dispersion from one host to another (Lindquist & Oldfield, 1996, Sabelis & Bruin, 1996).

According to the "Catalogue of the Eriophyoidea of the World", 13 nominal species of eriophyoid mites have

been registered on apple (*Malus × domestica* Borkh.): *Aculus schlechtendali* (Nalepa, 1890), *Aculus malivagrans* (Keifer, 1946), *Aculus malus* (Zaher & Abou-Awad, 1979), *Calepitrimerus baileyi* Keifer, 1938, *Calepitrimerus aphrastus* (Keifer, 1940), *Eriophyes mali* Nalepa, 1926, *Eriophyes malimarginemtorquens* Liro, 1951, *Epitrimerus pyri* (Nalepa, 1894), *Phyllocoptes mali* (Nalepa, 1926), *Phyllocoptes malinus* (Nalepa, 1895), *Rhinotergum schestovici* Petanović 1988, *Cecidophyes malifoliae* (Parrott, 1906) and *Diptacus gigantorhynchus* (Nalepa, 1892) (Amrine & Stasny, 1994). Five species have been registered on apple in the territory of Serbia to date: *A. schlechtendali*, *D. gigantorhynchus*, *E. pyri*, *P. malinus* and *R. schestovici* (Petanović & Stanković, 1999). *C. baileyi* has been registered recently (R. Petanović, database of Eriophyoidea of Serbia). Among them, only the apple rust mite (A. schlechtendali) is a widely distributed pest. Analyses made by Amrine (personal communication) have shown that A. malivagrans and A. malus are synonyms of A. schlechtendali, while C. aphrastus is synonym of C. baileyi, E. mali Burts is homonym of E. mali Nalepa, and P. mali (Nalepa, 1926) is synonym of E. mali Nalepa. Moreover, E. malimarginemtorquens is probably a valid species, but found very rarely, while *Epitrimerus pyri* (Nalepa) has been found on apple as an alternate host, and C. malifoliae is a very questionable species. Summarizing the list of species found on apple trees we may conclude that the apple rust mite (A. schlechtendali), apple blister mite (E. mali) and apple erineum mite (P. malinus) are pests of the apple, while *E. malimarginemtorquens*, *C.* baileyi, E. pyri, R. schestovici and D. gigantorhynchus can be its pests under certain circumstances.

The apple blister mite (*Eriophyes mali* Nal.) (Eriophyidae) has been recently registered for the first time in the territory of Serbia.

Distribution of *E. mali* has so far been recorded in North America, New Zealand, the European part of Russia, South Africa, Zimbabwe, and in many European countries (Belgium, Finland, Czech Republic, Poland, Germany, Hungary, Bulgaria, Slovenia, Bosnia and Herzegovina, and Croatia).

The apple blister mite was described for the first time by Nalepa (1926) as *Eriophyes pyri* var. *mali*, a new variety of Eriophyes pyri (Pgst. 1857). The mite caused similar symptoms on apple leaves as the pear blister mite on pear leaves. Type locality was not designated, although it has been suspected that it was in Austria (Amrine & Stasny, 1994). Later, Liro and Roivainen (1951) found the mite as the causative agent of blisters on apple leaves in Southern Finland. Blisters were greenish at the beginning and later became brown. The authors changed the mite's taxonomic status and raised it to the rank of species, i.e. *E. mali* (Nal.) Liro (nov. comb.). Burts (1970) then described a new species on apple, Eriophyes mali Burts, 1970, which can be considered as a junior homonym of *E. mali* Nal. (Amrine, personal communication). Manson (1984) detected the species in New Zealand. Considering the species to be E. mali Burts, he compared the morphological features of its New Zealand populations with the mites found by Burts in North Western America. Krasinskaya (1960) erroneously assigned the apple blister mite as Aceria and designated as Eriophyes (Aceria) mali. This designation means that the mite belongs to the genus Eriophyes, subgenus Aceria. In her paper, the biology of the mite, and seasonal changes in the density of its

females, males, nymphs and eggs were described, as well as the development of blister galls, their transformations from May to August and their relative abundance and distribution on leaves. Natchev (1978) made a list of eriophyoid mites on the apple in Bulgaria. Among other species, he described the appearance of galls of apple blister gall mites and gave a short description of the mite and a semischematic drawing. In his drawing of the prodorsal shield, the scapular setae (sc) are situated at the rear shield margin directed divergently to the rear, which is one of the diagnostic characters of the genus Aceria. The mite was designated as Aceria mali (Nal.). However, the lateral aspect of the anterior part of the mite clearly pointed at the position of *sc* tubercles in the position characteristic of the genus Eriophyes (Manson, 1984; Ueckermann, 1993). In a taxonomic study of the genus *Eriophyes* in Africa, Ueckermann (1993) cited the presence of *E*. mali Burts, giving only a description of symptoms and distinctive characters of the mite as compared to E. pyri.

The species *E. mali* was considered for a long time as a variety of the pear blister gall mite (*E. pyri*) because of its morphological similarities and the appearance of identical symptoms, blister galls on both apple and pear leaves.

Blister galls are made by the initial feeding of overwintering adult females. First symptoms of blister galls are small, red or pink swellings on the undersurface of leaves. Continued feeding of mites kills cells, causing the galls to change colour to dark brown or black on pear leaves and light brown on apple leaves. At the end of the vegetation season infested plant tissue becomes completely necrotic. Blister galls which are not infested by the first generation of mites are light green (Burts, 1970).

A detailed analysis of morphological characters of *E. mali* and *E. pyri* showed that they differed in the length of lateral, first ventral and second ventral setae. The setae are significantly longer on *E. pyri*. Additional features that are different in the two species are the body length and the number of opisthosomal annuli. *E. mali* is shorter and with fewer annuli (Burts, 1970; Manson, 1984). Moreover, Burts (1970) concluded from the experimental results that the species were host specific. Molecular methods (comparison of ITS1 and ITS2 regions of ribosomal DNA sequences) confirmed that the species were different (Kedzior et al., 2006).

As the species was detected for the first time in Serbia, and *E. mali* is an important pest, the paper presents the morphological characteristics important for species determination, symptoms and intensity of injury. Morphological characteristics of a population of *E. mali* from Serbia are compared with *E. pyri*, and with different populations of *E. mali* from remote regions of the world using literature data: the original description (Nalepa, 1926), and descriptions of a population from the USA (Burts, 1970) and a population from New Zealand (Manson, 1984).

MATERIALS AND METHODS

The presence of *E. mali* was detected in Serbia in an apple orchard in the suburbs of Belgrade (Ledine N 44º 48'17.2" E 20º 21' 34.6") in July of 2013. Mites were extracted using the method of de Lillo (2001). Permanent slides were made in Keifer's F medium using a modified method of Keifer (1975). Mites were observed under a phase contrast light optical microscope (Leica DMLS). Morphological characters of mites were measured using the software package IM 1000 (Leica, Germany), following instructions by Amrine and Manson (1996), and de Lillo et al. (2010). Thirty-four characters of 10 females were measured. All measurements are given in micrometers (μm) and, unless stated otherwise, refer to the length of structure. Direct measurements and the means were rounded up to the nearest integer. In order to obtain precise qualitative morphological characters, images were taken, using a scanning electron microscope (SEM, JEOL-JSM 6390) at the University of Belgrade, Faculty of Agriculture. Live mites were collected individually with a fine entomological needle from fresh plant parts under stereomicroscope and placed on a SEM holder.

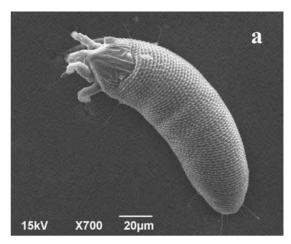
In addition, the percentage of surface of infested leaves covered with blister galls was calculated. Fifty leaves were chosen randomly and photographed, and leaf surface was measured using the computer program AutoCad (version 2012). Adobe Photoshop CS3 Extended Version program was used to determine the percentage of infestation.

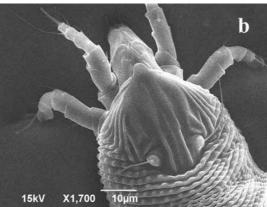
RESULTS AND DISCUSSION

Morphological characteristics of E. mali

Female (n=10) (Figure 1). Body light yellow and vermiform, 212 (199–220), 45 (42–50) width. **Gnathosoma** 22 (19–27) projecting obliquely downwards. **Prodorsal shield** 24 (21–27), 28 (25–32) wide, median line short, admedian lines complete and parallel, and several lateral lines. Scapular tubercles almost on rear shield margin, 14 (12–16) apart, scapular setae (*sc*) 19 (16–22), directed anteriorly. **Leg I** 34 (30–38); tibia 4 (3–5), tibial setae 5 (4–7); tarsus 4 (3–4); solenidion (ω) 7 (6–8); empodium (*em*) 6 (4–7), simple, 4-rayed. **Leg II** 29 (25–35); tibia 4 (3–5); tarsus 3 (2–3); solenidion (ω) 7 (6–8). **Opisthosoma** with 63–72 dorsal and 61-67 ventral annuli. Setae c2 17 (16–19) on annulus 9–11; setae d 19 (14–21) on annulus (20–24); setae e 8 (6–12) on annulus (37–40); setae f 20 (17–26) on 58-64 annulus. Setae h1 5 (4–6); h2 48 (30–79). Genitalia 13 (11–15), 18 (16–19) wide, coverflap with 9–12 longitudinal ridges, setae 3a 8 (6–9), 14 (13–15) apart.

Male: not seen.





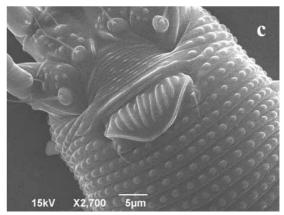


Figure 1. SEM photographs of *Eriophyes mali*. (a) dorsal view; (b) prodorsal shield; (c) coxigenital area (orig.).

E. mali closely resembles *E. pyri* but, according to comparisons made by Burts (1970) and Manson (1984), there are some characters important for their distinction. As it was stressed in Introduction, the most distinctive characters are: length of lateral (*c2*), first ventral (*d*) and second ventral setae (*e*), length of

body and the number of opisthosomal annuli. These morphometric characters are longer, and the number of annuli is higher in *E. pyri*.

The comparison made in our work (Table 1) shows that the most important distinctive character is the number of opisthosomal annuli, which is higher in E. *pyri*.

Characters	Eriophyes pyri USA	<i>Eriophyes pyri</i> New Zealand	<i>Eriophyes mali</i> Serbia
Length of body	200-230	135-195	212 (199–220)
Width of body	40-50	45-57	45 (42–50)
Length of gnathosoma	24	21-28	22 (19–27)
Length of prodorsal shield	26-28.5	26-32	24 (21–27)
Width of prodorsal shield	28.5	44-50	28 (25-32)
Length of setae <i>sc</i>	20-22	20-28	19 (16–22)
Tubercles of <i>sc</i> apart	12	10-13	14 (12–16)
Length of leg I	26-28	28-37	34 (30-38)
Length of genu	5		
Length of setae <i>l</i> "	17–19		
Length of tibia	5.5	6-8	4 (3-5)
Length of setae <i>l'</i>		6-8	5 (4-7)
Length of tarsus		6-8	4 (3-4)
Length of solenidion ω	7	5-7	7 (6-8)
Length of empodium <i>em</i>			6 (4–7)
Number of rays on empodium	4	4	4
Length of leg II	26-28	25-33	29 (25-35)
Length of genu	5		
Length of setae l''	7-8		
Length of tibia	5.5	5-6	4 (3-5)
Length of tarsus		6-8	3 (2-3)
Length of solenidion ω	8	7-9	7 (6-8)
Length of epigynium	10.5	8-14	13 (11–15)
Width of epigynium	21.5	19–21	18 (16–19)
Number of ridges			9–12
Length of setae <i>3a</i>	4.5	6–19	8 (6-9)
Apart of tubercles <i>3a</i>			14 (13–15)
Length of setae <i>c2</i>	14	20-38	17 (16–19)
On annulus	7	8	9–11
Length of setae <i>d</i>	14	17-65	19 (14–21)
On annulus	21	25	20 - 24
Length of setae <i>e</i>	4	7-14	8 (6-12)
On annulus	42	44	37-40
Length of setae <i>f</i>	16	22-28	20 (17–26)
On annulus	8 th rings from rear	7 th rings from rear	58-64
Number of opisthosomal annuli	90–95	71–113	61–72
Length of seta <i>h2</i>	40		48 (30–79)
Length of seta <i>h1</i>	5-6		5 (4-6)

Table 1. Comparison of characters of Eriophyes pyri (Keifer 1938 – USA; Manson 1984 – New Zealand) and Eriophyes mali (Serbia).

Comparing *E. mali* from different localities (Table 2), differences were evidenced between different populations regarding some morphological structures. The *E. mali* from Serbia is most similar to the *E. mali* described by Nalepa (1926). Both populations are characterized by almost the same body length, length of first and second legs, second ventral setae (*e*), and the number of striae on epigynium. *E. mali* from the USA and New Zealand populations are characterized by shorter body, first and second legs, and second ventral setae (*e*). The results of our analysis supports Amrine's (personal communication) decision to describe the species found in Indiana and West Virginia in the USA as *Eriophyes* n.sp. Mites from Serbia were also compared with some disposed characters of a Bulgarian population (Natchev, 1978). The *E. mali* from Bulgaria were: 201 μ m long, 51 μ m wide, had four rayed empodium, genital coverflap with 12 striae and opisthosoma with 78 annuli.

Characters	Nalepa 1926	USA	New Zealand	Serbia
Length of body	200	160	135-150	212(199-220)
Width of body	33	37.5	42-57	45 (42-50)
Length of gnathosoma	30	24	25-26	22 (19–27)
Length of prodorsal shield	32	24.5	21-25	24 (21–27)
Width of prodorsal shield		25	40-43	28 (25-32)
Length of setae sc	30	19.5	16-21	19 (16–22)
Tubercles of <i>sc</i> apart		13	12-14	14 (12–16)
Length of leg I	38	26	23-26	34 (30-38)
Length of genu		5		
Length of setae <i>l</i> "		18		
Length of tibia		5	5-6	4 (3-5)
Length of setae l'		18	4	5 (4-7)
Length of tarsus			5-6	4 (3-4)
Length of solenidion w	7.5	6–7	6	7 (6-8)
Length of empodium em				6 (4–7)
Number of rays on empodium	4	4	4	4
Length of leg II	29	26	22–25	29 (25–35)
Length of genu		5		
Length of setae <i>l</i> "		17-18		
Length of tibia		5	3-4	4 (3-5)
Length of tarsus			5-6	3 (2–3)
Length of solenidion ω	9.5	7	7-8	7 (6-8)
Length of epigynium		10.5	7–9	13 (11–15)
Width of epigynium	19	18.5	14-20	18 (16–19)
Number of ridges	9		8	9-12
Length of setae <i>3a</i>	12	7.9	5-9	8 (6–9)
Apart of tubercles <i>3a</i>				14 (13–15)
Length of setae c2	23	18.6	12–16	17 (16–19)
On annulus		7-8	8	9-11
Length of setae d	37	24.5	20-33	19 (14–21)
On annulus		20	23	20-24
Length of setae <i>e</i>	12	3.9	2-8	8 (6–12)
On annulus		37-42	37	37-40
Length of setae f	26	19.5	11–26	20 (17–26)
On annulus		7 th rings from rear	6 th rings from rear	58-64
Number of opisthosomal annuli		81	67-86	61–72
Length of seta <i>b2</i>		45		48 (30–79)
Length of seta <i>b1</i>	9	6		5 (4-6)

Table 2. Comparison of characters of Eriophyes mali from different localities (Nalepa 1926, USA, New Zealand and Serbia)

The comparison made in this study shows differences in the sizes of certain structures between the European and North American specimens. Namely, *E. mali* mites from Europe (Bulgaria [Austria?] and Serbia) differ from those of USA and New Zealand origin.

Symptoms and bionomy

E. mali overwinters at the base of buds or under bud scales. Females are the most numerous ovewintering mites (95-98%). Overwintering nymphs are very rare, while eggs and males were not found in buds. About 1000 individuals could be found in a single bud.

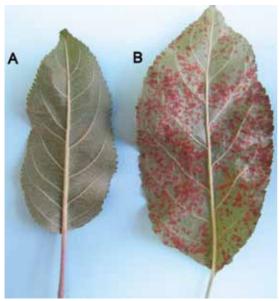


Figure 2. (A) - Healthy leaf; (B) - Infested leaf (orig.)

Mites start to disperse from buds from 8-18 May when average temperatures reach 11-17 °C. Females feed for two weeks on leaf surface and cause blister galls. Blisters can be found not only on leaves, but also on fruits. Females start laying eggs at the beginning of June, and larvae and nymphs appear (emerge) ten days later. Adults of summer generations emerge at the beginning of July. Males are less numerous in comparison with females, 2-5 males : 100 females. There are two generations per season. Second generation is more numerous and around 30 individuals can be found in one gall, while there are 18 individuals per gall in the first generation. One generation develops within 25-30 days. Females are present during the second summer generation (protogyne) and winter (deutogyne). Females start to search for overwintering places at the

end of July, but most of them go to overwinter in the middle and at the end of August (Krasinskaya, 1960).

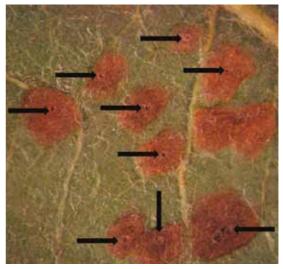


Figure 3. Blisters on apple leaf with openings at the centre (orig.)

Mite feeding creates blister gall formations on leaves (Figure 2). In the centre of each gall there is a hollow which mites enter and go out from (Figure 3). Later, mites feed and develop inside the gall (Figure 4).



Figure 4. *Eriophyes mali* within leaf blister gall (orig.)

The percentages of infested leaf surface ranged from 1.6% to 87.6%, the average being 22.4%. Infestation of most of the measured leaves was low with the average rate of 5%, while infestation exceeding 80% was only measured on two leaves (Figure 5).

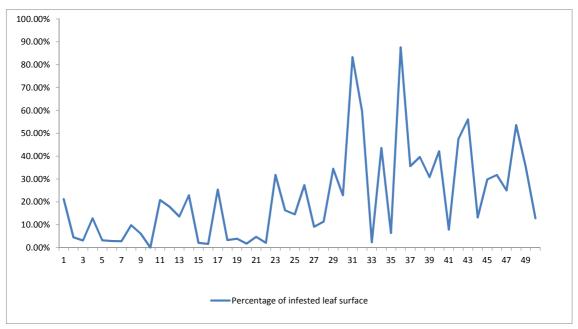


Figure 5. Range of percentage of infested leaf surface on fifty measured leaves

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Uporedna morfološka analiza eriofida plikastih gala lista jabuke, *Eriophyes mali* Nal., nove štetočine u Srbiji

REZIME

Eriofida plikastih gala lista jabuke - *Eriophyes mali* Nalepa, 1926 (Acari: Prostigmata: Eriophyoidea), po prvi put je registrovana kao nova štetočina jabuke na teritoriji Srbije. Prisustvo *E. mali* je do sada zabeleženo u Severnoj Americi, na Novom Zelandu, u evropskom delu Rusije, u Južnoj Africi, Zimbabveu i mnogim evropskim zemljama.

Zbog velike morfološke sličnosti i pojave istih simptoma na jabuci i krušci, dugo je *E. mali* smatrana varijetetom *E. pyri* – eriofide plikastih gala lista kruške. Do saznanja da se radi o različitim vrstama došlo se nakon detaljne analize morfoloških karaktera.

U radu je obrađen taksonomski status vrste *E. mali*, predstavljeni su rezultati analize morfoloških karaktera i stepena infestacije lisne površine.

Poređenjem *E. mali* sa različitih lokaliteta uočavaju se razlike u pojedinim morfološkim karakterima, koje mogu biti uslovljene njihovom geografskom distribucijom. *E. mali* iz Evrope se razlikuje od one iz USA i Novog Zelanda. *E. mali* iz Srbije je najsličnija populaciji *E. mali* koju je opisao Nalepa (1926) iz Evrope.

Usled ishrane *E. mali* biljnim sokovima po površini lista formiraju se gale u vidu plikova koje su na početku sitne, 2-3 mm, svetlo zelene boje, tokom vegetacije se povećavaju, a potom pocrne i osuše se. Procenat infestirane lisne površine se kretao od 1,6% do 87,6%, dok je prosečan procenat infestirane površine, izražen kao aritmetička sredina dobijenih vrednosti, bio 22,4%.

Ključne reči: Eriophyes mali; jabuka; Srbija