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Distribution and Occurrence of the Neogregarine Pathogen, *Ophryocystis anatoliensis* (Apicomplexa) in Populations of *Chrysomela populi* L. (Coleoptera: Chrysomelidae)

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Abstract. *Chrysomela populi* L. (Coleoptera; Chrysomelidae) is one of the most serious pests affecting poplars. Chemical control is the most widely known suppressive method against this pest. This method is not acceptable in urban forests because of their proximity to people. Entomopathogenic organisms are environmentally friendly control agents and suppress the pest populations under natural conditions. In the present study, the occurrence and distribution of a new entomopathogenic protist, a neogregarine, *Ophryocystis anatoliensis* (Apicomplexa) in *C. populi* populations and *a* member of the family Chrysomelidae is presented for the first time. In total, 90 of 2185 *C. populi* adults and larvae collected from 16 localities during three years (from 2013 to 2015) were found to be infected by this pathogen. Neogregarine infection was observed in 14 of 16 investigated *C. populi* populations. The infection was variable between the populations and years. These results confirm that the neogregarine pathogen has a high dispersal potential through the *C. populi* populations and can be an effective natural biological suppressing factor on the pest populations.

Key words: *Chrysomela* (= *Melasoma*) *populi*, Chrysomelidae, urban forest, neogregarine, *Ophryocystis anatoliensis,* biological control

INTRODUCTION

Poplars, including several rapidly grooving salicaceous trees of the genus *Populus* are commonly used plants for urban afforestation possessing several valuable characteristics which have led to multiple beneficial uses for society and the environment (Konijnendijk 2005; FAO 2009; Plotnik 2009; Isebrands and Richardson 2014). Despite their positive benefits in urban forestry and industry, multiple biotic risk factors affect the poplar breeding. The most important biotic factors hindering the efficiency of sustainable poplar breeding are pest insects damaging the plants (de Tillesse *et al.* 2007).

Chrysomela (= *Melasoma*) *populi* L. (Coleoptera; Chrysomelidae) is the most abundant and most important poplar pest species in Europe, frequently causing extensive damage in young poplar plantations and nurseries (Augustin *et al.* 1993; Urban 2006). The effect of this beetle is a reduction in biomass or the death of young nursery plants. To combat this beetle, chemical insecticides are applied, especially when its eggs begin to hatch (de Tillesse *et al.* 2007). Chemical control is

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the most widely known suppressive method but has many undesirable effects on the environment; it is especially unacceptable in urban forests because of their proximity to people. Control decisions for poplar pests, especially in urban areas and urban forests, should take into account ecological and social factors (de Tillesse *et al.* 2007). On the other hand, natural enemies are safe, sustainable and environmentally friendly control agents; they are highly host-specific, so other biotic and abiotic elements of the environment are unharmed (Undeen and Vávra 1997; Yaman *et al*. 2010; Wegensteiner *et al*. 2015). Therefore, ecological strategies for the control of *C. populi* infesting poplar trees in urban area have been desired (de Tillesse *et al*. 2007). Recently, it has become more important to study the natural enemies of *C. populi.* From this perspective, natural enemies of *C. populi* such as predators (Teodorescu 1980), parasitoids (Lotfalizadeh and Ahmadi 1998) and parasites (Tarasi *et al*. 2001) have been extensively studied and evaluated for controlling this pest (Teodorescu 1980; Zeki and Toros 1990; de Tillesse *et al*. 2007). However, there are a few records on the use of entomopathogens as a natural suppressing factor of *C. populi* (Sidor 1979; Sidor and Jodal 1986; Vriesen and Keller 1994) although coleopterans are frequently infected by entomopathogenic organisms (Poinar 1988; Takov *et al*. 2006; Theodorides 1988; Toguebaye *et al*. 1988; Yaman *et al*. 2008, 2010, 2011; Wegensteiner *et al*. 2015). Some entomopathogens such as nematodes (Rauther 1906; Jolivet and Théodoridés 1950), microsporidia (Sidor 1979; Sidor and Jodal 1986), fungi (de Tillesse *et al*. 2007; Assaf *et al*. 2012) and *Bacillus thuringiensis* (Vriesen and Keller 1994) have been studied to find possible ecological control agents against *C. populi,* but gregarines have yet to be studied. Among gregarines only the neogregarines have a high pathogenic effect on their hosts by destroying the host's fat body and exhausting energy sources. Recently, Yaman and Radek (2017) identified an entomopathogenic protist, *Ophryocystis anatoliensis* (Apicomplexa) in *C. populi* for the first time. It was the first record of *a* neogregarine species not only from *C. populi* but also from *a* member of the family Chrysomelidae. However there is not much knowledge on the effect of this pathogen on the *C. populi* populations in nature.

In the present study, the occurrence and distribution of the new neogregarine pathogen, *Ophryocystis anatoliensis* (Apicomplexa) in *C. populi* populations is presented for the first time by proposing an ecologically alternative biological control agent against this pest.

MATERIALS AND METHODS

Insect samples

Totally 2185 insects, 1712 adults and 473 larvae of *C. populi* were collected from geographically widely distributed sixteen localities in Turkey from April to September during the three-year (from 2013 to 2015) investigation (Fig. 1). They were kept at 24–28°C and 35–45% RH and 18:6 photoperiod in laboratory conditions till they were examined.

Sampled localities and dates:

Samsun; 17.04.2013 (Adults), 25.04.2014 (Adults)
Irmaksırtı (Samsun); 26.04.2013 (Adults), 03.06.2013 (Adults), 23.04.2014 (Adults), 20.07.2015 (Adults and Larvae)
Fidanlık (Kocaeli); 09.06.2013 (Adults), 02.05.2014 (Adults), 23.06.2014 (Adults)
Amasya; 16.05.2014 (Adults and Larvae)
Çankırı; 16.05.2014 (Adults and Larvae), 23.07.2015 (Adults and Larvae)
Bolu; 16.05.2014 (Adults)
Trabzon; 23.05.2014 (Adults)
Eskişehir; 19.05.2014 (Adults and Larvae)
Polatlı (Ankara); 19.05.2014 (Adults and Larvae)
Akyazı (Sakarya); 03.05.2014 (Adults and Larvae), 26.04.2014 (Adults and Larvae), 27.05.2014 (Adults and Larvae)
Bekdemir (Kastamonu); 19.04.2013 (Adults), 18.06.2014 (Adults), 23.06.2014 (Adults)
Alibeşe (Kastamonu); 30.06.2014 (Adults), 04.09.2014 (Adults and Larvae), 04.09.2014 (Adults)
Tosya (Kastamonu); 23.07.2015 (Adults and Larvae)
Tokat-Turhal; 14.07.2015 (Adults and Larvae)
Ankara-Kızılcahamam; 22.07.2015
Çorum; 21.07.2015 (Adults)
Karabük; 03.07.2015 (Adults)
Kırşehir; 22.07.2015 (Adults and Larvae)
Yozgat; 21.07.2015 (Adults and Larvae)

Microscopic examination

The adults and larvae were dissected in Ringer's solution by using dissection pins, and then prepared wet smears including host fat body, Malpighian tubules, gut epithelium and hemolymph were examined for presence of mature oocysts of the neogregarine under a light microscope at a magnification of 400–1000 ×. When an infection was found, the slides were air-dried and fixed with methanol, then stained with freshly prepared 5% solution of Giemsa stain. Giemsa stain was used to differentiate the neogregarine pathogen from other artifacts and resistant stages of other organisms and demonstrate the fine internal structure of the neogregarine pathogen and then re-examined under the microscope (Yaman and Radek 2003).

RESULTS AND DISCUSSION

During the three-year investigation we found neogregarine pathogens in several *C. populi* populations in nature. Neogregarine infection was confirmed by ob-

Fig. 1. Infected (bold) and non-infected localities where *Crysomela populi* adults and larvae were collected in Turkey.

(SC: Samsun-Çarşamba, SI: Samsun-Irmaksırtı, SV: Samsun-Vezirköprü, KO: Kocaeli-Fidanlık, AM: Amasya, CN: Çankırı, BO: Bolu, TR: Trabzon, ES: Eskişehir, PO: Polatlı, AK: Akyazı, KB: Kastamonu-Bekdemir, KA: Kastamonu-Alibeşe, ÇR: Çorum, KR: Kırşehir, YG:Yozgat, TK: Tokat, KR: Karabük, AN: Ankara).

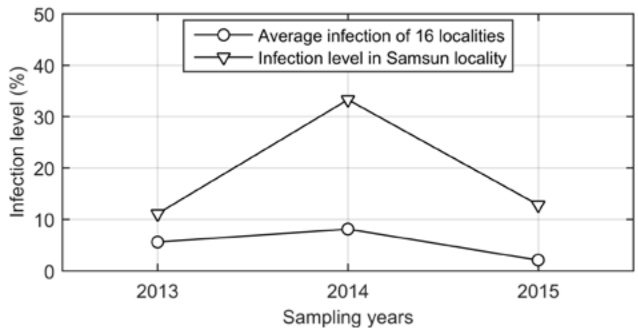

Fig. 2. *Ophryocystis anatoliensis* infection levels in *C. populi* populations in Turkey during the three years.

servation of the oocyst stages as an evidence of the infection. The typical characteristics of the oocysts were uniform, navicular shapes with plugs at the two poles as described by Yaman and Radek (2017). The neogregarine pathogen was observed frequently in the Malpigian tubules of *C. populi.* In total 2185 insects, 1712 adults and 473 larvae of *C. populi* were examined for any infection and 86 adults and 4 larvae of them were found to be infected by the pathogen (Table 1). The infection rate varied from 2.1 to 37.2%. The infection was variable between the populations and years. Total infection of all investigated populations was 5.6, 8.1 and 2.1% in 2013, 2014 and 2015, respectively. Total infection shows similarity with the infection in Samsun populations (Fig. 2). The infection was 11.1, 33.3 and 12.8% in Samsun locality in 2013, 2014 and 2015, respectively, in where the neogregarine infection was found for the first time by Yaman and Radek (2017) common. As seen in Figure 2, total neogregarine infection increased in 2014 and decreased again in the year 2015. These results also confirm that the pathogen can be able to suppress the populations as an alternative natural suppressing factor on *C. populi* populations.

Considering the pooled data of all three years, eighty-six of the examined 1712 adults were infected by the neogregarine pathogen and the average rate of infection for the adult beetles was 5.02%. Only four of the examined 473 larvae were infected by the neogregarine pathogen and the average rate of infection for the larvae was 0.9%. The infection was observed in fourteen adult and two larval populations of the 16 investigated localities. 87.5% of the investigated localities were found to be infected by the neogregarine pathogen and that is accepted as high prevalence. The neogregarine pathogen was observed in fourteen of the 16 investigated adult populations of *C. populi.* When compared with the adult infection, the larval infection was 5.9 times less on average (Table 2). Furthermore, the maximum infection level was observed as 37.2% in the adults and 5% in larvae. On the other hand, there is statistically significant difference in the infection levels of adult and larval individuals of *C. populi* (Pearson Chi-square, P: 0,000 < 0.05). These results support our hypothesize that *C. populi* larvae are more sensitive than adults to the neogregarine pathogen. Lord (2007) found that the larvae of *Cryptolestes ferrugineus* are more susceptible than adults, and most of the infected larvae do not survive to complete development. This is important to decide which life stage should be started to struggle using the pathogen.

As seen in Figure 1, the neogregarine infection is widespread in *C. populi* populations in Turkey. Wide distribution of the neogregarine pathogen presented here may be evidence that the pathogen has high dispersal potential through *C. populi* populations. Such potential is a desirable characteristic for a biological control agent (Pereira *et al*. 2002).

Yaman and Radek (2017) identified the neogregarine pathogen presented here as a new species *Ophryocystis anatoliensis* (Apicomplexa) in *C. populi* and it was also the first record from a member of the chrysomelids. When compared with eugregarines, neogregarines are more virulent pathogens so their infection is often lethal (Undeen and Vavra 1997). Here we focus on the occurrence and distribution of the neogregarine patho-

Table 1. Occurrence of neogregarine pathogen, *Ophryocystis anatoliensis* in the populations of *Chrysomela populi* (Coleoptera: Chrysomelidae)

Locality	Sampling date	Number of the examined sample		Number of the infected sample and infection rate (%)			
		Adults	Larvae	Adults	%	Larvae	%
Samsun-Çarşamba	17.04.2013	28	–	0	0	–	–
Samsun-Vezirköprü	25.04.2014	12	–	2	16.6	–	–
Samsun-Irmaksırtı	26.04.2013	24	–	5	20	–	–
	03.06.2013	11	–	2	22	–	–
	23.04.2014	51	–	19	37.2	–	–
	20.07.2015	47	70	6	12.7	1	1.4
Kocaeli-Fidanlık	09.06.2013	30	–	0	0	–	–
	02.05.2014	38	–	7	18.4	–	–
	23.06.2014	45	–	4	8.8	–	–
Amasya	16.05.2014	21	31	3	14.3	0	0
Çankırı	16.05.2014	40	60	6	15	3	5
	23.07.2015	22	–	0	0	–	–
Bolu	16.05.2014	27	–	3	11.1	–	–
Trabzon	23.05.2014	11	–	0	0	–	–
Eskişehir	19.05.2014	60	71	0	0	0	0
Polatlı	19.05.2014	51	39	0	0	0	0
Akyazı	03.05.2014	18	108	3	16.6	0	0
	26.04.2014	31	–	3	9.6	–	–
	27.05.2014	12	–	1	8.3	–	–
Kastamonu-Bekdemir	19.04.2013	68	–	0	0	–	–
	18.06.2014	170	–	4	2.3	–	–
	23.06.2014	46	–	1	2.1	–	–
Kastamonu-Alibeşe	30.06.2014	61	–	7	11.4	–	–
	04.09.2014	50	29	0	0	0	0
	04.09.2014	35	–	0	0	–	–
Kastamonu-Tosya	23.07.2015	29	9	0	0	0	0
Tokat-Turhal	14.07.2015	137	29	2	1.4	–	–
Ankara-Kızılcahamam	22.07.2015	107	–	0	0	–	–
Çorum	21.07.2015	75	–	3	4	–	–
Karabük	03.07.2015	70	–	2	2.8	–	–
Kırşehir	22.07.2015	149	23	2	1.3	0	0
Yozgat	21.07.2015	136	4	1	0.7	0	0
Total		**1712**	**473**	**86**	**5.02**	**4**	**0.85**

Table 2. Infection of *Chrysomela populi* with *Ophryocystis anatoliensis*. Comparison of the infection grades of adults and larvae

Life stage	Populations			Individual		
	Examined	Infected	%	Examined	Infected	%
Adult	16	14	87.5	1712	86	5.02
Larvae	10	2	20	473	4	0.85

gen, *Ophryocystis anatoliensis* to confirm whether the pathogen presented here would be an alternative natural suppressing factor in *C. populi* populations from several localities in Turkey.

C. populi is known as an insect pest in the most abundant and most important species for forestry and it occurs in almost all countries of Europe (Urban 2006). However, there are few studies on natural entomopathogenic organisms suppressing its populations. Until now, a microsporidian pathogen, *Nosema melasomae* (Sidor and Jodal 1986), different *Bacillus thuringiensis* isolates (Vriesen and Keller 1994) and an entomopathogenic fungus, *Paceilomyces farinosus* (Assaf *et al.* 2012) and a neogregarine, *C. anatoliensis* (Yaman and Radek 2017) have been studied as potential control agents against this pest.

The use of entomopathogens against poplar pests in urban forests has been very limited. There are a few studies on using living microorganisms for the control of harmful insects on poplars. Biological control of poplar pests (especially in urban forests) is an alternative to using chemical pesticides and very important ecologically (de Tillesse *et al*. 2007) because poplars are one of the most frequently used plants for urban afforestation. Therefore the results of this study on an important poplar pest, *C. populi* are of great importance to the search for safe and effective agents for biological control of poplar pests in urban areas.

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