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Monochamus galloprovincialis distribution in Aleppo pine forests in Tunisia

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Abstract: The pine wilt disease, caused by the pine wood nematode (PWN) *Bursaphelenchus xylophilus*, was detected in Europe in 1999 in Portugal and the longhorn beetle *Monochamus galloprovincialis* reported as the only vector since 2001. Although not present in northern Africa, it is feared that the PWN may cause significant damage if introduced into the Maghreb region, where several susceptible pine species which can serve as hosts are found, along with insects of the *Monochamus* genus which can act as vectors. In order to assess the risk of propagation of the wilt disease, we surveyed for the presence of possible vectors of the *Monochamus* genus in Tunisia, characterizing the distribution and emergence pattern. Studies were carried in nine locations with Aleppo pine (*Pinus halepensis*) forests. Sampling for insects was based on the trap tree technique, allowing beetles to lay eggs in the field and subsequently emerging. We confirmed the presence of *Monochamus* beetles in Tunisia, with only one species detected, *M. galloprovincialis*, which was widespread in the Aleppo pine forests. Our results show that this specie can develop and emerge from the basal, median and the upper part of the Aleppo pines with similar success. The larval development took nearly one year and adult emergences occurred from May to August during 2012. Results are discussed in view of similar biological studies conducted in other Mediterranean countries and the implications for the risk assessment of pine wilt disease in Tunisia.
Keywords: Pine sawyer, *Pinus halepensis*, Emergence, Xylophagous beetle, Pine wilt disease

Monochamus galloprovincialis'in Tunus'taki Halep çamı ormanlarında yayılışı

Özet: Çam odun nematodu *Bursaphelenchus xylophilus*'un yol açtığı çam solgunluk hastalığı, Avrupa'da 1999 yılında Portekiz'de tespit edildi ve teke böceği *Monochamus galloprovincialis* 2001 yılından bu yana tek vektör olarak bildirildi. Kuzey Afrika'da var olmasa da nematodun, konukçu olarak kullanılabilecek çeşitli hassas çam türlerinin yer aldığı Mağreb bölgesine girmesi halinde, *Monochamus* cinsinin vektör olarak hareket edebilecek böcekleriyle birlikte önemli bir hasara yol açmasından korkulmaktadır. Solgunluk hastalığının yayılma riskini değerlendirmek amacıyla, Tunus'ta yayılış ve çıkış özelliklerini belirleyerek *Monochamus* cinsindeki olası vektörlerin varlığını araştırdık. Çalışmalar Halep çamı (*Pinus halepensis*) ormanlarında dokuz bölgede gerçekleştirilmiştir. Tuzak ağaç tekniğiyle böcek örnekleri toplanmıştır ve böylelikle böcekler bu sahada yumurtlamış ve ardından çıkmıştır. Tunus'ta *Monochamus* cinsinden sadece tek bir türün varlığını, Halep çamı ormanlarında yaygın olan *M. galloprovincialis* türünü tespit ettik. Sonuçlarımız, bu türün Halep çamlarının taban, orta ve üst kısımlarında benzer başarı oranlarıyla gelişebileceğini ve çıkabileceğini göstermektedir. Larva gelişimi yaklaşık bir yıl sürmektedir ve erginlerin çıkışı 2010 yılında Mayıs ve Ağustos ayları arasında gerçekleşmiştir. Sonuçlar, diğer Akdeniz ülkelerinde yürütülen benzer biyolojik çalışmalar ışığında ve Tunus'ta çam solgunluk hastalığının risk değerlendirmesinin olası sonuçları açısından değerlendirilmiştir.
Anahtar kelimeler: Çam odun zararlısı, *Pinus halepensis*, Çıkış, Oduncul böcek, Çam solgunluk hastalığı

1. Introduction

In Tunisia, Aleppo pine (*Pinus halepensis*) is the most common pine specie, covering almost 54% of the forested area (DGF, 2010). Despite its importance, it is characterised by serious regeneration constrains, mainly because of anthropic pressure and over-exploitation. It is also suffering from attacks by various insect pests, namely bark and wood borers such as *Tomicus destruens*, *Orthotomicus erosus* and *Pityogenes calcaratus* (Ben Jamâa, 2007), but also from new invasive pests (Ben Jamâa et al., 2013), which are implicated explicitly or implicitly in the pine forests decline. The pine wood nematode (PWN) *Bursaphelenchus xylophilus*, and its vectors of *Monochamus* genus are nowadays among the most feared invasive pests in Europe and northern Africa, as these two agents are able to wilt and kill several susceptible species of pines (Mota et Vieira, 2010). In Europe, the nematode and its vector have been

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studied in detail since the introduction of the PWN in 1999, and the most important features of the insect's bio-ecology and its interaction with the nematode are now well known (Naves et al., 2006, 2007, 2008; Akbulut et Stamps, 2011). The same cannot be said for the southern side of the Mediterranean sea, as with the exception of isolated notes on the presence of *M. galloprovincialis* in Northern Africa, including Tunisia (Hellrigl, 1971), no detailed studies have been conducted until now, despite the serious risk to this area due to the widespread presence of pine hosts, vectors and significant commercial trade of wood with countries where the nematode is already present. In this paper we begin to characterize the presence of the pine sawyer *M. galloprovincialis* in Tunisia, its distribution in the Aleppo pine forests and its emergence pattern.

2. Materials and methods

The study was carried out in nine representative Aleppo pine (*Pinus halepensis*) forests in Tunisia (Table 1), belonging to the humid, subhumid, semi arid and arid superior bioclimate. The survey of *Monochamus* was conducted with the trap tree technique. Two healthy pine trees were cut at 20 cm from its base in each site from July to august 2011 and left in the field to allow the oviposition of beetles. The whole trees were kept on the soil at a shaded place. The two trees are distant with about 10 m in each forest.

After 43 days, three logs of 1m of length were taken from each tree in each forest. One log from the base, one from the medium and the last one's from the upper part of each tree (N= 54 logs), and taken to the INRGREF Entomological Laboratory in Tunis, where they were kept separately in an insect-proof bags under natural conditions. Indeed, the INRGREF station is belonging to the semi arid bio-climate. In this station, the average of the annual temperature reach the 18.1°C (T° min: 4.9°C; T° max: 36.5°C) and the precipitation didn't exceed the 500 mm (Auge et Francelet, 1970).

All the logs stored in the INRGREF station were weekly monitored until September 2012. Emerging adult insects were collected, counted, identified and conserved in alcohol (95%). Insect identification was made at the Entomological Laboratories of INRGREF in Tunisia and of INIAV in Portugal, using several keys (Picard, 1929; Vives, 2000). Voucher specimens are kept in the INRGREF entomological collection in Tunis, Tunisia.

Kruskal–Wallis nonparametric analysis of variance test was used to compare the mean number of emerged beetles between localities, bioclimates and tree sections, with $\alpha = 0,05$. Statistical procedures were performed using the SPSS software.

Table 1. Location of the Aleppo pine (Ap) forests studied in Tunisia. Sp – Stone pine (*Pinus pinea*), Mp – Maritime pine (*Pinus pinaster*)

Districts	Forests	GPS coordinates	Bioclimate Classification (CNEA, 2007)	Stands nature	Pines species
Bizerte	Azib	N37°12,862'; E09°58,332'	Humid	Mixed	Ap, Sp , Mp
Jendouba	Dar fatma	N36°49,090'; E08°44,795'	Humid	Mixed	Ap, Sp , Mp
Nabeul	Oued Bir	N36°53,354'; E10°47,896'	Subhumid	Mixed	Ap, Sp
	Darchichou	N36°57,725'; E10°59,514'	Subhumid	Mixed	Ap, Sp
Zaghouan	Sidi Aouidet	N36°22,69'; E09°76,450'	Semi-arid	Pure	Ap
Siliana	Sidi Said	N36°11,623'; E09°39,140'	Semi-arid	Pure	Ap
Kef	Kebouche	N36°12,773'; E08°54,566'	Semi-arid	Pure	Ap
Kasserine	Samama	N35°20, 14'; E08°48,320'	Arid superior	Pure	Ap
	Ain Amara	N35°16,41'; E08°30,000'	Arid superior	Pure	Ap

3. Results

3.1. Presence and distribution of the pine sawyer beetles

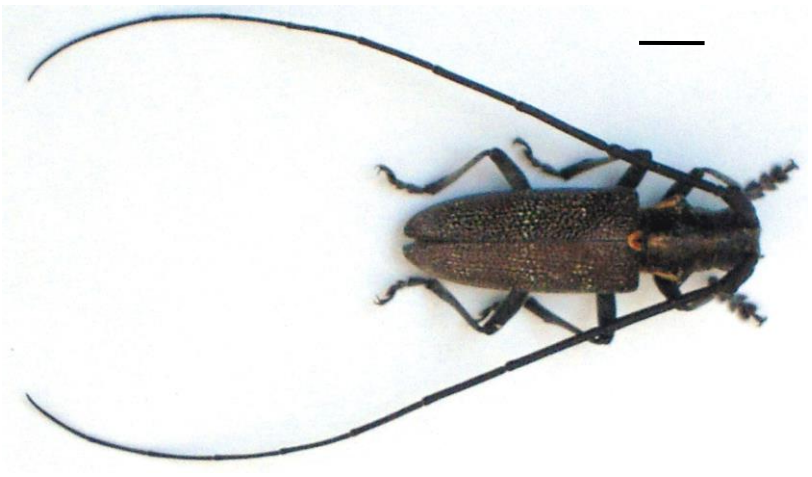

Figure 1. Adult male of *Monochamus galloprovincialis.* Scale bar = 1 cm

During this study, the presence of *Monochamus galloprovincialis* Olivier 1795 in the Tunisian Aleppo pine (Ap) forests was confirmed (Figure 1), this being the only species of its genus detected. This pine sawyer was found in all sampled localities, from central to northern Tunisia (Figure 2).

The symptoms of the feeding and oviposition of the longhorn beetles on the bark or wood of the felled pines were very conspicuous and clearly noticeable in the field (Figure 3).

Logs of trap-trees from Samama, Central Tunisia, where the most colonized by *M. galloprovincialis*. Indeed, these logs contain almost 39% of the total number of the emerged beetles, followed by Oued Bir and Kebouche forests, each with 16% of the total. Inversely, logs from the Azib and Dar Fatma forests (Northern Tunisia), and belonging to the humid bioclimatic area, had the lowest number of emergences, with just 4% each (Table 2). These variations resulted in significant differences for the mean number of emerged beetles between localities (Kruskal–Wallis test: $\chi 2 = 22.2$, d.f. $= 8$, $p = 0.045$), and also between the different bioclimatic area (Kruskal–Wallis test: $\chi 2 = 6.8$, d.f. $= 3$, $p = 0.077$).

The mean number of *M. galloprovincialis* emerging per pine log was 19 adults, with a maximum of 62 in Oued Bir region (Nabeul: Northern Tunisia) and a minimum of 2 in Azib region (Bizerte: Northern Tunisia) (Table 2). Comparing the emergence by tree sections, no significant differences were found on the number of *M. galloprovincialis* from the upper, medium and basal parts of the trap-trees (Kruskal–Wallis test: $\chi 2 = 3.31$, d.f. $= 2$, $p = 0.1915$), although a slightly higher number of beetles emerged from the middle section of the trees

Figure 2. Tunisian localities were *M. galloprovincialis* was detected (1: Azib; 2: Darchichou; 3: Oued Bir; 4: Dar fatma; 5: Kebouche; 6: Sidi Aouidet; 7: Sidi Said; 8: Samama; 9: Ain Amara)

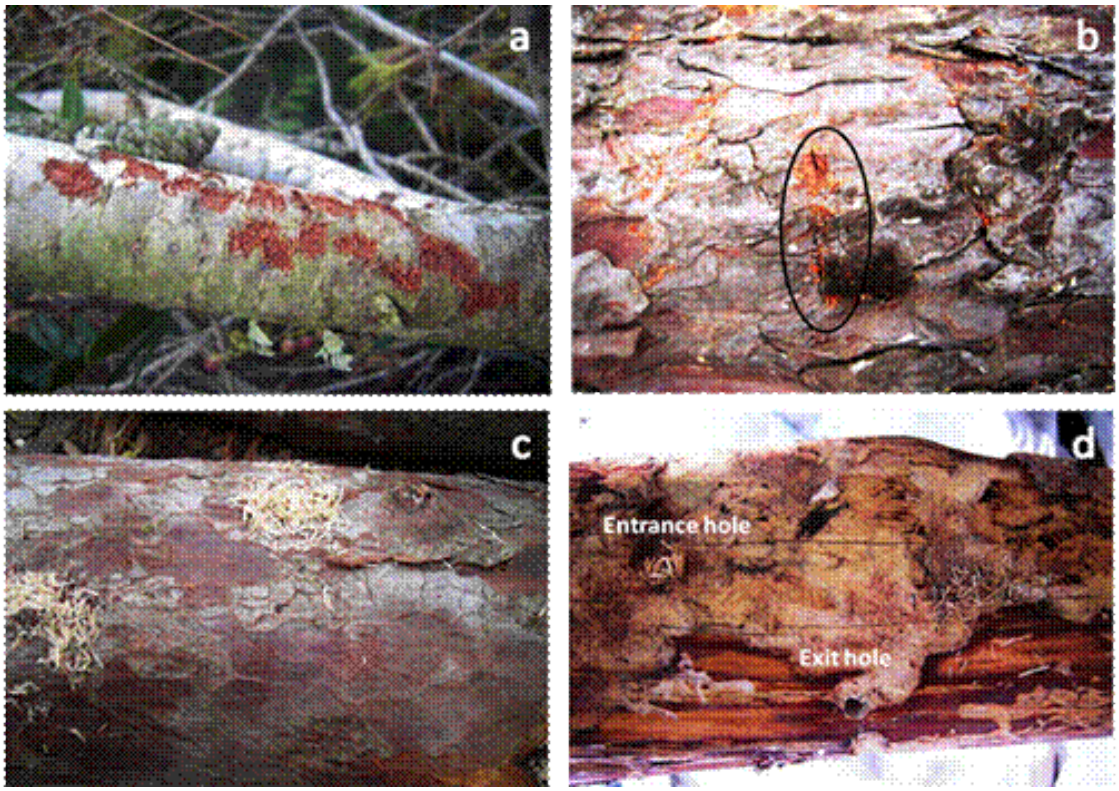

Figure 3. Evidence of *M. galloprovincialis* presence on Aleppo pine (a: nutritional wounds on the bark, b: eggs notches, c: sawdust of larval stages; d: larval galleries in the wood)

Table 2. Emerged beetles from localities in Tunisia (mean ± SE). * Means in the column with the same letter do not differ significantly, Multiple Comparison test

Forests	Location in Tunisia	Bioclimatic Area	Trap tree diameter at 1.30 m (cm)	Emerged beetles		
				Number	%	Insects per log*
Azib	North	Humid	6.05	41	4.07	6.8±1.7a
Dar Fatma	North	Humid	5.57	38	3.77	12.7±4.7a
Darchichou	North East	SubHumid	8.99	93	9.24	15.5±2.4ab
Oued Bir	North East	SubHumid	5.81	164	16.30	27.3±8.7ab
Sidi Aouidet	Center	SemiArid	7.24	129	12.82	21.5±4.3ab
Sidi Said	Center	Semi arid	6.44	66	6.56	11.0±3.0a
Kebouche	Center	Semi Arid	5.33	165	16,40	27.5±3.9ab
Ain amara	Center	Arid superior	7.88	74	7.35	12,3±3.7a
Samama	Center	Arid superior	8.28	236	23.46	39.3±2.3b
			Total number of emerged beetles	1006	100	18.62±3.8

3.2. Emergence pattern

No cerambycid emerged during 2011. Emergencies only occurred during the subsequent year, 2012, and therefore *M. galloprovincialis* required one year completing its life cycle in Tunisia. The 1006 adults, obtained from the stored logs in the INRGREF, emerged during the spring-summer period (Figure 4), with the earliest beetle emerging in mid–May (from Samama), while the latest beetles appeared in august from Oued Bir logs. This emergence seems to be correlated to the temperature (R^2= 0.594). According to our observations, and considering the origin of each log, the peak of emergence of *M. galloprovincialis* in Tunisia occurs during the June- July period (Figure 5).

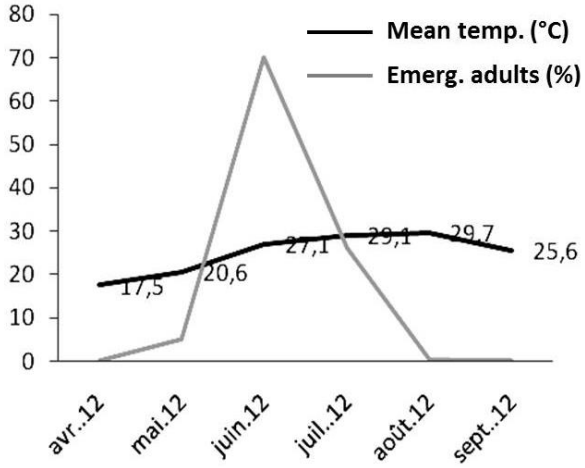

Figure 4. Emergence pattern of M. galloprovincialis from stored Aleppo pine logs at the INRGREF station

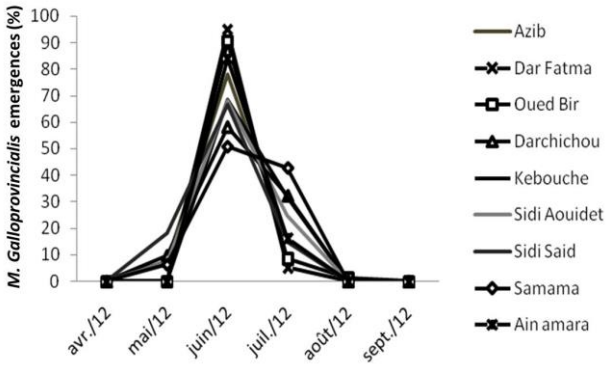

Figure 5. Emergence pattern of M. galloprovincialis from logs of each localities.

4. Discussion

Despite the recognized importance of *M. galloprovincialis* as one of the main vector's of PWN in Europe (Naves et al., 2007, Akbulut and Stamps, 2010), no previous study had been done on its presence and distribution in the Maghreb region in North Africa, with the expectation of scattered records on its presence (Hellrigl, 1971; Kerris and Guerroudj, 1991). In Tunisia, both the *Monochamus* species and *B. xylophilus* were classified as quarantine pests (Jort, 2012).

Our results confirm the presence of *M. galloprovincialis* on Aleppo pine on Tunisia, in accordance to previous references by Hellrigl in 1971. The pine sawyer is native to Tunisia and is the only *Monochamus* species found in the country (Mejri et al., 2014: in press), as well as in northern Africa (Hellrigl, 1971). Our results confirm that it is widely distributed in Tunisia, following the distribution of its main host plant, Aleppo pine. If we assume that the number of eggs laid in the trap-trees is related to the abundance of insects in the forest, we can conclude that the insect´s abundance differs between localities, with contrasting patterns between the arid (more abundant insect populations) and semi-arid (less abundant) locations. Nevertheless, other factors beside the climatic conditions of each location might be involved, because in the arid zones the forests consist on monocultures of just one pine species (Aleppo pine), while in the humid locations other pines, such as maritime pine *P. pinaster*, can also be found in abundance, and can serve as alternative host for the insects and attract egg-laying female beetles.

Concerning the biology of the insect, ours results illustrate that in Tunisia, like in similar Mediterranean countries, *M. galloprovincialis* presents one generation per year (Tomminen, 1993; Francardi and Pennachio, 1996; Koutroumpa, 2007; Naves et al., 2008, Akbulut et al., 2008). Unfortunately, we couldn't study the biological traits of the insect and its larval development in each locality and in each bioclimatic area because of some field constraints. However, the only difference will be in the duration of the larval development in the localities belonging to the arid superior bioclimatic area. Indeed, *M. galloprovincialis* will emerge earlier in those localities comparing to the semi arid, subhumid and humid ones. The pattern of adult emergence is also quite similar to other locations from Europe, namely from Italy (Francardi and Pennachio, 1996) and Portugal (Naves et al., 2008), with extended emergence periods during several months in late spring/summer, but with emergence peaks in June and July. This is a preliminary assessment on the distribution and basic biological parameters of the pine sawyer *M. galloprovincialis* associated with Aleppo pine in Tunisia. Future studies should address the insect´s association with other possible host available in the country, such as *Pinus pinaster* and *Pinus pinea*, and its biology in these pines and on other locations. Additionally, research on the association of the pine sawyer with nematodes in Tunisia is also being conducted, and will constitute a valuable tool in assessing the risk of introduction of the Pine wood nematode into this northern Africa country.

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