GEOGRAPHIC SPREAD AND INTER-ANNUAL EVOLUTION OF THE POPULATIONS OF *Trioza erytreae* IN THE IBERIAN PENINSULA

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Abstract

Trioza erytreae is *per se* a major citrus pest, although its destructive power is much greater as a vector of the bacteria causing the most devastating disease of citrus worldwide: the citrus huanglongbing (HLB). Following early detections of *T. erytreae* in mainland Europe, simultaneously in Portugal and Spain, its distributional range has rapidly increased, thus becoming a serious threat to the entire Mediterranean citrus industry. One year after our lastest survey, the situation has only worsened, with the vector pest being around 200 km closer to key citrus growing areas in Southern Portugal and Spain. Once *T. erytreae* invades a new area, the degree of severity of its attacks increases very quickly, which is indicative of its potential for invasiveness. We highlight that *T. erytreae* is currently spread and well established along the Northwestern coast of the Iberian Peninsula, ranging from Cedeira in A Coruña (Spain) to Pontes/Setúbal in Portugal, covering approximately 600 km on a straight uninterrupted line. Implementation of further containment and control measures are critical to addressing this growing concern.

Keywords

African citrus psyllid; huanglongbing; '*Candidatus* Liberibacter spp.'; *Citrus* spp.; Spain; Portugal

Trioza erytreae (Del Guercio, 1918) (Hemiptera: Triozidae), commonly referred to as the African citrus psyllid, is one of the two known vectors of the bacteria causing the huanglongbing disease (HLB), currently deemed as the most devastating disease affecting citruses. The other one is the Asian citrus psyllid, *Diaphorina citri* Kuwayama, 1908 (Liviidae). Bacteria causing this vascular disease are three phloem-limited, non-cultured, Gram-negative '*Candidatus* Liberibacter spp.': '*Ca*. Liberibacter americanus', '*Ca*. Liberibacter asiaticus' and '*Ca*. Liberibacter africanus', *T. erytreae* being the natural vector of the African HLB.

This psyllid only feeds on Rutaceae and has a special predilection for lemon trees, although all commercial and economically important citrus species may be affected (Arenas-Arenas et al 2018). Recently, rutaceous host plant species have demonstrated to affect morphometric parameters in adults, with those specimens reared on *C. limon* and *Citroncirus* spp. (Citrumelo) being largaer, followed by *C. sinensis* and *Murraya koenigii*, which is often related to have better fitness parameters and potential for dispersion (Owusu et al 2018).

The African citrus psyllid harbors a great capacity to damage *Citrus* spp. plantations as a pest. Each gravid female is capable of laying thousands of eggs in a single season, preferably on the edges of the leaves and along the central nerve (Fig. 1). After coming out of the egg, nymphs begin feeding the phloem sap, thereby producing characteristic open gall-like structures on leaves, which are unequivocal signs of their presence (Cocuzza et al 2016). Each gall corresponds to a single nymph nest and is characterised by a globular distortion on the upper side of the leaf,

corresponding to a concave hollow at the lower part, where the nymph inhabits until it completes its immature development (Fig. 2). Nonetheless, all nymphal stages are mobile and can move towards other leaves or even other shoots when conditions are adverse, where they induce new galls (pers. obs.). It is also worth emphasizing that as a result of the high herbivore pressure, new shoots become irregular and yellowish. Moreover, adults and all nymphal stages produce large amounts of honeydew whilst feeding that falling on leaves and fruits, thus promoting sooty mold development on them. On the other hand, adults have a great dispersal ability and can readily invade new areas located several hundred meters away (van den Berg 1990). Despite all this, the worst damage is caused when it acts as a vector of HLB (Bové 2006).

T. erytreae is believed to be native of Southern Africa, where the earlier records date from 1897 (van den Berg 1990). Over the last century, it spread throughout the entire length of the Eastern coast of Africa (including Madagascar, Mauritius and Reunion islands), from where it colonised the Arabian Peninsula in Asia (Yemen and Saudi Arabia) and many countries of Central Africa (Angola, Cameroon and Democratic Republic of the Congo). The earliest detections in Europe were in islands and non-mainland systems, first in Madeira (1994) and later in the Canary Islands (2002), where it is currently widely spread. Since *T. erytreae* was detected in continental Europe in summer 2014, specifically in the Western part of both the Pontevedra province (Spain) and Porto (Portugal) (Pérez-Otero et al 2015), its presence and distribution has increased dramatically. In just three years, from 2014 to 2017, it spread over 400 km of the Western coastline in the Iberian Peninsula, from San Xiao (Spain) to Maiorca (Portugal) (Arenas-Arenas et al 2018).

Here below, we report the results of a recent survey assessing the current status of the populations of *T. erytreae* in the Iberian Peninsula conducted in summer 2018, and covering from Figueira da Foz to Coimbra and the Portuguese western coastline from Figueira da Foz to Quarteira. Additionally, we integrated these results with those published by both the Direção-Geral de Alimentação e Veterinária (DGAV) and the Galician Government (Xunta de Galicia) (DOG No 119 2017), the competent authorities to carry out the periodical monitoring of *T. erytreae* in Portugal and Galicia (Spain), respectively.

The sampling took place at the end of July 2018. We first considered the area between Figueira da Foz to Coimbra, a particularly sensitive area due to its role as a leading producer of citrus seedlings. After that, we proceeded in a north-south direction until Ferreira Do Alentejo (the most Southerly surveyed location in the Alentejo region). Lastly, we also surveyed the area surrounding Quarteira in order to rule out the existence of isolated outbreaks in the Algarve region (around 150 km away from Ferreira Do Alentejo), which is also deemed a high-risk area because it has traditionally been a sweet-orange producing region.

As with our previous survey (Arenas-Arenas *et al*., 2018), in order to assess population density, a citrus tree was deemed to be affected by *T. erytreae* when a single psyllid or a gall was observed (even empty galls). The severity

of the attacks caused by the pest was estimated using the following scale:

- 0: No evidence of *T. erytreae* adults, nymphs or nests.
- 1: No evidence of *T. erytreae* adults nor evidence of the nymphs, but typical empty nests present (prior infestation occurred).
- 2: Low level of infestation, with only 1-2 leaves in the whole tree showing evidence of the presence of *T. erytreae* (Fig. 2).
- 3: Considerable level of infestation, with a high number of shoots affected and with large numbers of galls per leaf.
- 4: Widespread level of infestation, with almost all shoots being affected and with leaves full of galls.

In the first monitored area from Figueira da Foz to Coimbra, *T. erytreae* spread inwards only a little over ten kilometers in comparison to the previous year (Fig. 3). Nevertheless, higher numbers of more severe outbreaks were identified this time, in Figueira da Foz, Carritos, Casal de Areia (degree 3 in *C. limon*, *C. sinensis* and *C. reticulate* trees), Maiorca (degree 1) or Montemor-o-Velho (degree 2) (see Table 1 for more information). In the prior survey conducted in this area, Maiorca was found to be the innermost infected point, whereas on this occasion it was Montemor-o-Velho, only 30 km west of Coimbra.

On the other hand, in the area between Figueira da Foz and Pontes/Setúbal, virtually all of the assessed localities recorded positive trees, although it is noteworthy that the degree of severity decreased towards the South (Table 1). In contrast, none of the municipalities assessed in the Alentejo region showed evidence of occurrence of the pest: Alcácer Do Sal, Santa Margarida Do Sado, Figueira Dos Cavaleiros or Ferreira Do Alentejo (Table 1 and Fig. 3). Nor was any sign found in Quarteira, thereby ruling out the existence of isolated outbreaks in the Algarve region for the present. Therefore, Pontes/Setúbal was the most southerly locality found to be affected in this survey as well the most Southern locality with *T. erytreae* being present in the Iberian Peninsula. This means that the pest moved more than 200 km southward in just one year (June 2017 – July 2018).

These results show that *T. erytreae* is currently present in the area between Figueira da Foz and Lisbon, from where it crossed the Tajo River and reached the Northern part of the Estuary do Sado (Pontes/Setúbal). Even more importantly, the species is currently distributed uninterruptedly from the Northerly to the Southerly known localities (Cedeira in A Coruña, Spain and Pontes in Portugal, respectively), covering approximately 600 km on a straight line since the first record in 2015. Furthermore, in accordance with the inter-annual evolution of *T. erytreae* populations in the Iberian Peninsula, once *T. erytreae* invades a new area, the degree of severity of their attacks rapidly increases.

While HLB is apparently absent in all countries in the Mediterranean basin, including Madeira and the Canary Islands (CABI, 2018), the growing distributional range of one of its natural vectors in Portugal and Spain has triggered

a phytosanitary emergence in both countries, but particularly in Spain, the leading producer of citrus in the area (with annual production exceeding 5 million tones over the last decade). Contingency measures include a prohibition on the movement of citrus plants from infected areas, the obligation to propagate young rutaceous plants only under greenhouse conditions (for nurseries embedded in demarcated areas such as those in Coimbra), and regular pest vector monitoring and chemical control. Chemical-based control strategies against *T. erytreae* in Europe are based on neonicotinoid insecticides as active substances. However, neonicotinoids (with the exception of acetamiprid) have recently been banned in the EU (Regulation No 2018/785 of 29 May 2018), and their outdoor usage has severely been restricted by their proven toxicity for bees foraging in nectar or pollen in succeeding crops. This restriction will come into force in December 2018. Given the high potential for invasiveness of *T. erytreae* and because of its proximity to key citrus growing areas in Southern Portugal and Spain, further efforts should be exerted in order to facilitate a speedy resolution and to enhance current control strategies.

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158**LEGENDS TO THE FIGURES**

159**Fig. 1** Several *T. erytreae* individuals simultaneously laying thousands of eggs in a single shoot.

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161**Fig. 2** Colony of *T. erytreae* on the bottom side of the leaf with many nymphs protected inside the galls.

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163**Fig. 3** Map showing the current status of *T. erytreae* in the Iberian Peninsula. White points: distribution in Galicia 164(Spain), published in the DOG No 119 of June 23, 2017. Blue points: distribution in Portugal, published by DGAV in 165August 10, 2018. Yellow points: outbreaks recorded in our previous surveys in summer 2016 (August) and 2017 (June), 166published in Arenas-Arenas *et al*. (2018). Red points: new outbreaks in Portugal in summer 2018 (end of July). Source 167image: Google Earth.

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187**Table 1.** Studied localities throughout the two-year survey. Table showing the year (2016, 2017 or 2018), geographical
188section and specific locality (from north to south), citrus species where *T. erytreae* was found, degree of damage (from 0
189to 4) and other observations.

YEAR	SECTION	LOCALITY	CITRUS SPECIES ASSESSED	I
2016	San Xiao - Freixo (A Coruña)	San Xiao	*C. limon*	
		Camariñas	*C. limon*	
		Tufións	*C. limon*	
		Cebráns	*C. limon, C. aurantium*	
		Vilar de Sobremonte	*C. limon, C. aurantium*	
		Muxia	*C. limon*	
		Frixe	*C. limon, C. aurantium*	
		Fisterra	*C. limon, C. sinensis*	
		Sardiñeiro	*C. limon*	
		Cee	*C. aurantium*	
		Ézaro	*C. limon, C. aurantium*	
		Carnota	*C. limon*	
		Sofan	*C. limon*	
		Lira	*C. limon*	
		Muros	*C. limon, C. sinensis*	
		Tal	*C. limon; C. sinensis*	
		Freixo	*C. limon, C. sinensis*	
		Quintáns	*C. aurantium*	
	Camos - Tabagón (Pontevedra)	Camos	*C. aurantium*	
		Nigrán	*C. limon, C. reticulata, C. aurantium*	
		Baiona	*C. aurantium*	
		A Guarda	*C. limon, C. sinensis*	
		Pías	*C. limon, C. aurantium*	
		Tabagón	*C. limon, C. aurantium*	
	Vila Nova de Cerveira - Aveiro (Portugal)	Vila Nova de Cerveira	*C. limon, C. sinensis*	
		Moledo	*C. limon, C. sinensis*	
		Âncora	*C. limon, C. aurantium*	
		Carreço	*C.aurantium, C. reticulata*	
		Viana do Castelo	*C. limon, C.aurantium*	
		Darque	*C. limon, C. sinensis, C. reticulata, C.paradisi*	
		Santana	*C. limon*	
		Esposende	*C. limon, C. sinensis, C. reticulata, C.paradisi*	
		Apúlia	*C. limon, C. reticulata, C. aurantium*	
		Estela	*C. limon*	
		Navais	*C. limon*	
		Amorim	*C. limon, C. reticulata, C. aurantium*	
		Vila do Conde	*C. limon*	
		Mindelo	*C. limon*	
		Porto	*C. sinensis, C. aurantium*	
		Sobral/Ovar	*C. limon*	
		Aveiro	*C. limon*	

2017	Aveiro - Figueira da Foz	Aveiro	*C. limon, C. reticulata*
		Gafanha do Carmo	*C. limon, C. sinensis*
		Gafanha da Boa Hora	*C. limon, C. sinensis, C. reticulata*
		Poço da Cruz	*C. sinensis*
		Praia de Mira	*C. sinensis*
		Mira	*C. limon*
		Cabeço de Mira	*C. limon, C. sinensis*
		São Caetano	*C. sinensis*
		Tocha	*C. limon, C. sinensis*
		Bon Sucesso	*C. limon*
		Ervedal (Quiaios)	-
		Tavarede	*C. sinensis*
		Figuiera da Foz	-
	Figueira da Foz - Coimbra	Vila Verde	*C. limon, C. sinensis, C. aurantifolia*
		Maiorca	*C. aurantium, C. limon, C. sinensis*
		Montemor-o-Velho	*C. limon, C. sinensis*
		Meãs	*C. limon, C. sinensis, C. reticulata*
		São Martinho de Árvore	*C. limon*
		São João do Campo	*C. sinensis, C. aurantium, C. paradisi*
		Coimbra	*C. sinensis*
	Lisbon and Setúbal	Montijo	*C. aurantium*
		Jardia	*Citrus spp., Fortunella spp.*
		Setúbal	*C. sinensis, C. aurantium*
2018	Figueira da Foz - Coimbra	Figueira da Foz	*C. limon*
		Carritos	*C. sinensis, C. reticulata*
		Casal Da Areia/Caseira	*C. sinensis, C.limon*
		Casal do Mato/Carvalhal	*C. limon*
		Maiorca	*C. sinensis*
		Montemor-o-Velho	*C. limon*
		Ameal	*C. limon*
		Assafarge	*C. limon, C. sinensis, C. reticulata*
		Coimbra	*C. sinensis, C. reticulata, C.aurantium, C.limon*
	Figueira da Foz - Quarteira	Amor	*C. limon, C. aurantium*
		Barreiros (Amor)	*C. limon, C. reticulata, C. sinensis*
		Pero Neto	*C. reticulata*
		Marinha Grande	*C. limon, C. reticulata*
		Valado Dos Frades	*C. sinensis, C. limon*
		Pederneira (Nazaré)	*C. sinensis, C.limon*
		Caldas Da Rainha	*C. limon, C. aurantium, C. sinensis*
		Obidos	*C. reticulata, C. limon*
		Amoreira	*C. sinensis*
		Olho Marinho	*C. sinensis, C. reticulata*
		Casal Do Alto Foz	*C. reticulata*
		Lourinha	*C. sinensis, C. reticulata*
		Torres Vedras	*C. sinensis, C. reticulata*
		Fonte Do Rei	*C. sinensis*
		Mafra	*C. limon*
		Sintra	*C. limon*

Almada	*C. sinensis*
Fogueteiro	*C. sinensis*
Setúbal	*C. limon, C. sinensis, C. reticulata*
Pontes/Setúbal	*C. limon, C. reticulata*
Alcácer Do Sal	*C. sinensis*
Santa Margarida Do Sado	*C. sinensis, C. limon*
Figueira Dos Cavaleiros	*C. sinensis*
Ferreira Do Alentejo	*C. limon, C. sinensis*
Quarteira	*C. sinensis, C. limon*