IS THE PRESENCE OF *Trioza erytreae*, VECTOR OF HUANGLONGBING DISEASE, ENDANGERING THE MEDITERRANEAN CITRUS INDUSTRY? SURVEY OF ITS POPULATION DENSITY AND GEOGRAPHICAL SPREAD OVER THE LAST YEARS

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Running title: *Trioza erytreae* in the Iberian Peninsula

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Summary

Huanglongbing (HLB) is by far the most devastating disease of citrus crops across the world, because it promotes a progressive weakening of citrus trees (until death in around ten years) that also involves a sudden loss of their production capacity. The African psyllid *Trioza erytreae*, one of the two known vectors of the bacteria causing HLB, is present in the northwestern Iberian Peninsula only since 2014. A previous survey done in 2015 showed a considerably spread both towards the north and the south, so we proposed a large-scale survey in the subsequent two years in order to know its current distributional range, population density and, thus, the effectiveness of contingency plans. Our results evidence a growing number of new affected areas throughout the Iberian Peninsula as well as higher severity of its attacks, being currently present from San Xiao (Spain) to Maiorca (Portugal), over 400 kilometers of coastline. The greater ability of *T. erytreae* to reach new areas may be due to the existence of higher outbreaks and population densities, what represents a serious threat for the nearing citrus producing areas. If no further control and management measures are implemented, it seems unavoidable the *T. erytreae* spread across Europe.

Key words

Candidatus Liberibacter spp.; *Citrus* spp.; degree of severity; Spain; Portugal

Psyllids (Hemiptera: Psylloideae) belong to a diverse group of small phytophagous insects closely related to aphids and white flies, whose action determines a typical set of symptoms to a wide range of cultivated plants, such as pear or olive trees (Casado *et al*., 2016). In recent years, scientists and agricultural agencies have paid special attention to these arthropods due to their role as vectors of diseases in crops of economic interest, as is the case of citrus crops. In this regard, the Asian citrus psyllid, *Diaphorina citri* Kuwayama, 1908 (Liviidae), and the African citrus psyllid, *T. erytreae* (Del Guercio, 1918) (Triozidae), are vectors of bacteria belonging to the genus '*Candidatus* (*Ca*.) Liberibacter spp.' (Koch's postulates have not been fulfilled yet), which is associated with the

disease called citrus huanglongbing (HLB) (citrus greening), by far the most devastating citrus disease worldwide (Duran-Vila and Bové, 2015).

Nowadays, HLB bacteria, vectors and susceptible citrus genotypes are present in most citrus growing regions of the world, with only Australia and the Mediterranean Basin remaining still HLB-free (Duran-Vila and Bové, 2015). However, recent events have raised the alarm in both the Spanish and Portuguese citrus industry. In summer 2014, the first record of the African citrus psyllid took place in both Galicia (northwester Spain) and northern Portugal (Pérez-Otero *et al*., 2015), representing the first time that one HLB's vectors was found in a country of the Mediterranean Basin. The western coast of the Iberian Peninsula presents hundreds of citrus trees located in small scattered orchards and gardens, as well as a suitable climate for the development of the African citrus psyllid, what eventually could become a way for the HLB spread to the nearest citrus growing areas: the Algarve (Portugal) and western Andalusia (Spain). On the other hand, current pest control programs and contingency plans for *T. erytreae* are not giving accurate solutions to this problem, together with the fact that citrus growers and particular gardeners have no training to recognise the pest or they are not fully aware of the harmful effects inherent to both the pest and the disease. Taking into consideration that after the vector arrival to certain area usually occurs the bacteria arrival [(Bové, 2006); (Duran-Vila and Bové, 2013); (Duran-Vila and Bové, 2015)], increased efforts should be done for containing the spread of this pest.

The presence of *T. erytreae* can be easily detected in the field because their nymphs are only found on the underside of leaves, where their activity promotes the formation of typical galls, each one corresponding to a nymph nest. Each nest 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 their immature development. Once it happens, the adults leave the nests forever, but empty hollows remain clearly visible, being this fact really useful for the early diagnosis of *T. erytreae* (Bové and Duran-Vila, 2016).

Here we report the results of our two-years survey assessing the current status of the

populations of *T. erytreae* in the Iberian Peninsula, which covered a large part of the western coastline of Galicia and northern Portugal: from Muros and Noia estuary, where *T. erytreae* was previously detected in 2015 (Bové and Duran-Vila, 2016), to Setúbal towards the south (Portugal) and to San Xiao towards the north (Spain).

This survey was carried out along the western coastal region of the Iberian Peninsula, considering two consecutive years: 2016 and 2017. The first sampling took place in August 2016 and covered from western Galicia to the north of Portugal. It started in Freixo, in the north side of the Muros and Noia estuary (A Coruña, Spain), from where we conducted the samplings both towards the north and the south. The second one was conducted in June 2017 and it was almost exclusively done along the northwestern coastline of Portugal. It started in Aveiro, proceeding in a north-south direction until arriving to Figueira da Foz, from where we moved inward until Coimbra. In order to discard the existence of other isolate outbreaks of the pest in southern regions of Portugal, we additionally surveyed the area surrounding both Lisbon (estuary do Tejo) and Setúbal (estuary do Sado), which are considered as high-risk areas because of their significant freight traffic of citrus fruits and seedlings and because of their proximity to key citrus growing areas in the south of Portugal and Spain.

Samplings were conducted in small commercial orchards as well as in isolated trees located in public or private gardens (see Table). We had into account different types of citrus cultivars, including all the citrus species present in the studied area (in decreasing order of abundance): lemon (*Citrus limon*), sweet orange (*Citrus sinensis*), mandarin (*Citrus reticulata*), sour orange (*Citrus aurantium*), grapefruit (*Citrus paradisi*) and lime (*Citrus aurantifolia*), in this way being able to compare with those results obtained in a previous survey conducted by Bové and Duran-Vila in 2015.

To assess the population density throughout the survey, we considered that a citrus tree was affected by *T. erytreae* when a single psyllid individual or gall was observed, even if the galls were empty. The severity of the attacks caused by the pest was estimated according to our own damage

assessment scale, which ranged from 0 to 4. The various degrees of severity mean the following:

- 0: There is no evidence of *T. erytreae* adults or their nymphs.
- 1: There are neither evidence of *T. erytreae* adults nor evidence of the nymphs, but typical empty nests are presented (prior infestation occurred) (Figure 1).
- 2: There is a low level of infestation, with only 1-2 leaves in the whole tree being infested (Figure 2A).
- 3: There is a considerable level of infestation, with a high number of shoots being infested and with a large number of galls per leaf (Figure 2B).
- 4: Widespread level of infestation, with almost all shoots being infested and with leaves full of galls (Figure 2C).

The increasing expansion of *T. erytreae* in the Iberian Peninsula was clearly evidenced after the last survey carried out by Duran and Bové in August 2015, when they reported its distributional range from Fisterra (A Coruña, Spain) to Porto (northern Portugal), also highlighting two locations with high degree of infestation: the Muros and Noia estuary, and the mouth of the Miño River (Figure 3). For this reason, we took the municipality of Freixo in A Coruña Province (in the north side of the Muros and Noia estuary) as starting point of our survey in August 2016, conducting the samplings both towards the north and the south in order to determine whether there has been an increase (expansion) or decrease (contention) in the range of distribution of *T. erytreae* (Figure 4), as well as to assess its population density.

In the **first phase of our survey** (August 2016), we found that *T. erytreae* had spread to many areas towards the north, being San Xiao the most northerly point, about 200 km north of Fisterra, where summer shoots of two lemon trees presented high level of incidence (degree 3 of damage). We also found *T. erytreae* in young shoots of lemon and sweet orange trees at intermediate locations between Freixo and San Xiao, which presented different degrees of severity. In Lira, Sofan, Carnota and Fervenza do Ézaro, positive trees presented an intermediate level of infestation (degree 2), whereas in Muros, Tal, Sardiñeiro de Abaixo and Fisterra the extent of the

infestation was the highest (degree 4). Moreover, in Fisterra we found a greater population density compared to the previous year (August 2015), with a noticeable increase in the number of galls both per leaf and per shoot. On the contrary, several coastal (Mugía and Camariñas) and inland (Tufións, Quintáns, Cebráns and Frixe) municipalities did not presented evidence of infestation (degree 0) (see Table for more information).

Regarding the samplings conducted towards the south of Freixo and along the entire coast of the Pontevedra Province, we found that the degree of severity and the affected citrus tree species varied according to the municipality studied (Table). The most commonly affected species were lemons, followed by sweet oranges, as occurred in Camos, A Guarda and Pía, where we recorded a degree 2 of damage, or Bayona and Tabagón, where we found a degree 3 of damage. By other side, we found the highest degree of incidence (degree 4) throughout the Council of Nigrán, where mandarin trees were also found to be affected by *T. erytreae*.

With reference to the samplings performed in Portugal, we found differences among localities in both the citrus tree species affected and the degree of severity again. Vila Nova de Cerveira, in the south side of the Miño River, was the northerly locality assessed, where we found that spring shoots of several lemon and sweet orange trees presented low level of infestation (degree 1), whereas summer shoots were not affected. On the contrary, in the mouth of the Miño River, particularly in Moledo, young shoots of these two citrus tree species were highly infested in both summer and spring outbreaks (degree 4). As we moved towards the south, another municipalities were found to be affected, including both lemon and sweet orange trees, such as Âncora (degree 2), Carreço (degree 2), Viana do Castelo (degree 2), Darque (degree 2), Marinhas (degree 4), Esposende (degree 4, also found in grapefruit trees), Apúlia (degree 3, also found in mandarin trees), Estela (degree 2), Navais (degree 2), Amorim (degree 2), Vila do Conde (degree 2), Mindelo (degree 2), Porto (degree 3), Sobral and Ovar (degree 2), and Aveiro (degree 3). No evidences were found in other southern places, that is, Aveiro was the southerly locality affected in the first phase of the survey.

The **second phase of the survey** (June 2017) was resumed in Aveiro, the southerly point at which the pest was present in the preceding year. This time we found the highest degree of damage in both lemon and mandarin trees located in Aveiro (degree 4), in which there were present higher numbers of eggs, nymphs and adults per shoot in comparison to 2016. As the survey progressed towards the south, we found another locations being affected: Gafanha do Carmo (degree 2 in both lemon and mandarin trees located in a mixed orchard), Gafanha da Boa Hora (degree 2 in lemon, sweet orange and mandarin trees), Poço da Cruz (degree 1, only in two sweet orange trees) and Mira (degree 3 in one lemon tree) (Figure 5). From that point onwards, no other locations were found to be affected, such as Cabeço de Mira, São Caetano, Tocha, Bon Sucesso and Tavarede (Table).

Because the proximity of Coimbra, a leader producer of citrus seedlings and a substantial exporter of a great variety of citrus fruits, we also took the area between Figueira da Foz and Coimbra into consideration. Only in Maiorca we found one isolated sour orange tree likely affected by *T. erytreae*, which showed very recent galls with no signs of nymphs or adults, being this the southerly point in which *T. erytreae* was recoded; whereas the other studied localities were free: Vila Verde, Montemor-o-Velho, Meãs, Tentúgal, São Martinho de Árvore, São João do Campo, and Coimbra (Figure 5). Nor did we find any evidence of the pest in the area surrounding Lisbon and Setúbal, such as in Montijo, Jardia (tree nursery) or the municipality of Setúbal, thereby ruling out the existence of other isolate outbreaks of the pest in southern regions of the Iberian Peninsula. See Table for more detailed description of each mentioned location.

Considering that Porto was the southerly affected locality in 2015 (Durán and Bové, 2016), the information presented here confirms that *T. erytreae* is currently spreading towards the south at high speed. This pest is already spread from San Xiao (Spain) to Maiorca (Portugal), for some 450 km of coast between Spain and Portugal, so in just two years *T. erytreae* has reached many new areas and with higher population density, suggesting higher spreading speed than in previous years, which is probably due to higher and more geographically dispersed number of outbreaks.

Furthermore, Maiorca is just a few kilometers from Coimbra (around 30) and to approximately 400 km of the main citrus producing in Spain and Portugal, what could represents a serious threat for many other producing areas. If no further control and management measures are implemented, it seems unavoidable the *T. erytreae* spread throughout the Iberian Peninsula.

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Figure 1: Underside of an orange leaf showing empty nests, here considered as degree 1 of damage. Modified imagen of that which was published by J. Bové in 2015.

Figure 2: Degrees of damage 2 (A), 3 (B) and 4 (C). In the figure 2A we can see a shoot presenting nymphs inside the nests. In the 2B, a large number of eggs and galls in a fresh shoot of lemon tree. In the 2C, single adult of *T. erytreae* on the upper side of citrus leaves presenting many galls. Authors: F.J. Arenas-Arenas and A. Hervalejo.

Figure 3: Status of *T. erytreae* in the northwest coastline of Spain and Portugal in 2015. Source image: Google Earth.

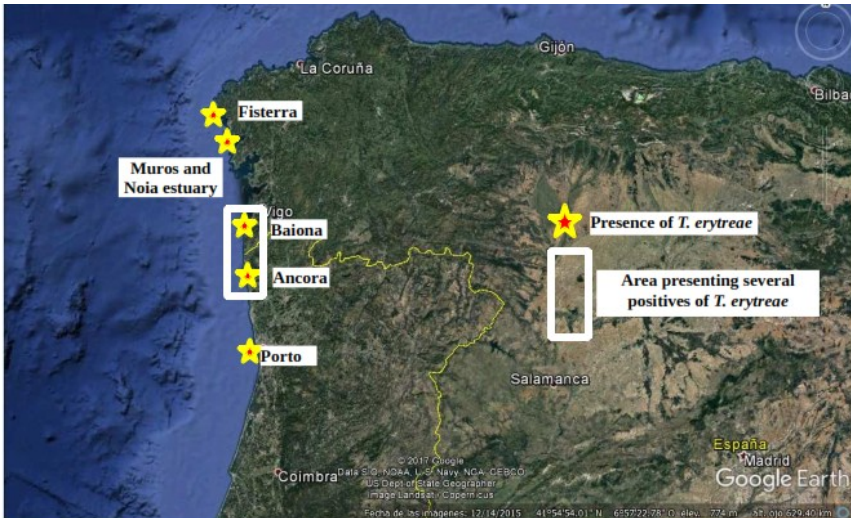

Figure 4: Status of *T. erytreae* in the northwest coastline of Spain and Portugal in August 2016. Source image: Google Earth.

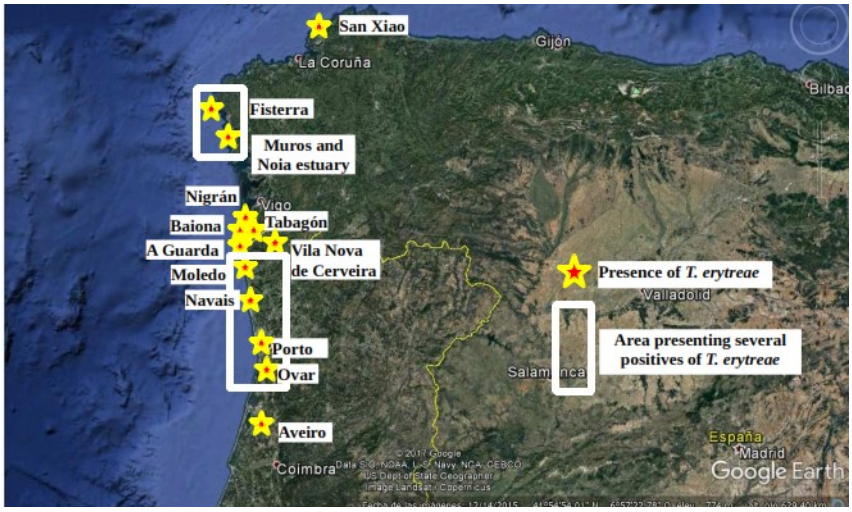

Figure 5: Status of *T. erytreae* along the Portuguese coastline, from Figueira da Foz to Coimbra, and in the Lisbon and Setúbal estuaries. Source image: Google Earth.

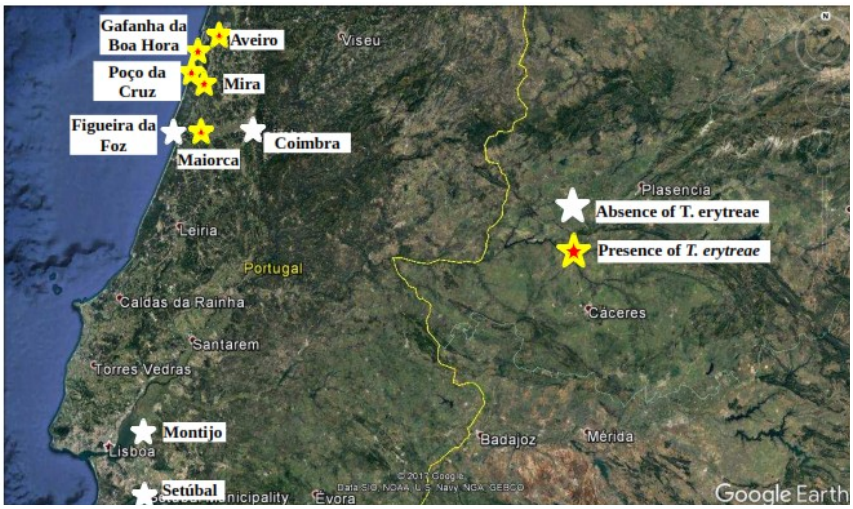

Table: Studied localities throughout the two-year survey.

YEAR	SECTION	LOCALITY	CITRUS SPECIES ASSESSED	D
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 Árvo-re	*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*

Table showing the year (2016 or 2017), geographical section and specific locality (from north to south), citrus species were *T. erytreae* was found, degree of damage (from 0 to 3) and other observations.