Development of a PCR-based method for the screening of potential predators of the African citrus psyllid *Trioza erytreae* (Del Guercio)

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Abstract

Trioza erytreae is one of the vectors of Huanglongbing (HLB), the main global citrus groves threat. Since its recent detection in the north-western Iberian Peninsula (Spain and Portugal), its contention and eradication have been a priority to prevent its spread. For the biological control of *T. erytreae,* it is important to understand the role that each potential natural enemy could have. With the aim to determine which predators have incorporated *T. erytreae* into their diet, a PCR-based method has been developed for the specific detection of *T. erytreae* in their gut contents. For this, a pair of specific primers was designed from the mitochondrial cytochrome c oxidase subunit I (COI) region. Specificity of this pair of primers was studied and feeding trials with two predator species were conducted to determine the decay rates of *T. erytreae* within their gut. None of the non-target species was amplified, showing the high specificity of these *T. erytreae* primers. Feeding trials showed 4.8h and 4.5h half-life time detections of *T. erytreae* ingested by *Chrysoperla carnea* and *Cryptolaemus montrouzieri*, respectively. Finally, field-collected generalist predators of *T. erytreae*-infested citrus trees from the Canary Islands and Galicia (Spain), were analysed by conventional PCR for the presence of *T. erytreae* in their guts. Results showed that a wide range of predator taxa ingested the target prey, like the families Coccinellidae, Anthocoridae, Chrysopidae, Hemerobiidae, Forficulidae, Miridae, Syrphidae, Formicidae, Erythraeidae and the order Araneae, with detection percentages ranging from 20 to 100%. These results confirm that most of the analysed generalist predators found in citrus trees could be potential candidates for the biological control of *T. erytreae* in future biological control programs of this HLB vector.

Key words: biological control; citrus; African citrus psyllid; gut-content analysis; ants; specific primers.

1. Introduction

The African citrus psyllid, *Trioza erytreae* (Del Guercio) (Hemiptera: Triozidae) is a pest mainly known for being one of the main vector species of Huanlongbing (HLB) (Urbaneja et al., 2020), the main global threat for citrus groves (Halbert and Manjunath, 2004), as the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae). While *D. citri* is native from the South of Asia, *T. erytreae* is a south-east African native species that has been present in Madeira (Portugal) and Canary Islands (Spain) since 1994 and 2002, respectively (Carvalho and Aguiar, 1997; González-Hernández, 2003). In mainland Europe, it was firstly detected in the north-western Iberian Peninsula (Galicia) in 2014 (Pérez-Otero et al., 2015). Then, it was rapidly spread along the Atlantic coast from the North to the South of Portugal, and along the Cantabrian coast from the West to the East of Spain (EPPO, 2020), threating the Spanish citriculture located in the South of the country and in the Mediterranean coast. Although Spain is nowadays free of HLB (Wang, 2020), the contention and eradication of *T. erytreae* is extremely important to reduce transmission risks under the potential HLB presence (Urbaneja-Bernat et al., 2020).

Until now, *T. erytreae* has been mainly managed with chemical treatments with a high frequency of broad spectrum pesticide applications per season, but its control has not been successful (Cocuzza et al., 2017; Gottwald, 2010). Chemical control of this pest is difficult to combine with integrated pest management (IPM) strategies in citrus groves in Spain, because of the high number of pesticide applications, which could negatively affect natural enemies, disrupting biological control strategies being applied for the management of various pests (Jacas and Urbaneja, 2010). Moreover, the fact that *T. erytreae* is present in private and urban gardens, make even more necessary a less harmful alternative.

Several polyphagous predators have been described to be potentially useful to manage *T. erytreae* in citrus groves in South Africa, as the families Coccinellidae, Anthocoridae, Miridae, Chrysopidae, Hemerobiidae, Syrphdiae and Formicidae, together with the order Arachnida (Catling, 1970; van den Berg et al., 1987). In the Canary Islands, the presence of some of them (coccinellids, anthocorids, lacewing and spiders) in citrus trees infested with *T. erytreae* has also been reported, but even if some species of these families had been observed feeding on *T. erytreae* (Estévez et al., 2018; González-Hernández, 2003), the real impact of these predators in the field, avoiding laboratory artifacts, remains still unknown. Even if they have contributed to reduce *T. erytreae* populations, they have not succeeded to contain it. A successful classical biological control of this pest was achieved in Reunion Island with the host-specific parasitoid *Tamarixia dryi* (Waterson) (Hymenoptera: Eulophidae) (Etienne and Aubert, 1980). For this reason, *T. dryi* was introduced in Tenerife (Canary Islands, Spain) in spring of 2018, showing ratios of parasitism higher than 70% (Hernández-Suárez et al., 2020) and rapidly spreading to other Canary Islands, as Gran Canaria. After that, *T. dryi* was released in three sites in Pontevedra (Galicia, Spain) in

autumn of 2019 and in spring and summer of 2020. Until now, the parasitoid has spread more than 30 km, and up to 75% of parasitism rate has been reported (Tena et al., 2021).

Given the risk of the potential arrival of *T. erytreae* to the main Spanish citrus area, the biological control of *T. erytreae* is necessary to be approached in several aspects and, in particular, to find out which native generalist predators where the pest is currently located better contribute to reduce this psyllid populations. A suitable approach for identifying predator-prey interactions in an agroecosystem is the use of molecular markers with prey-specific primers for gut content analysis of generalist predators (Agustí et al., 2003a). This method has been previously used to study trophic relationships between some predators and some citrus pest, like *Forficula auricularia* L. (Dermaptera: Forficulidae) to feed on aphids (Romeu-Dalmau et al., 2012), or several predator species to feed on the California red scale *Aeonidella aurantii* (Maskell) (Hemiptera: Diaspididae) (Bouvet et al., 2019).

In the present study, a *T. erytreae*-specific pair of primers has been designed and a conventional PCR protocol has been developed for the detection of *T. erytreae* within field-collected generalist predators. This tool allows tracking *T. erytreae* frequencies of predation by the predator assemblage present in citrus groves in Spain under natural field conditions, showing potential candidates for biological control further strategies.

2. Material and methods

2.1. Primer design, DNA extraction and amplification

A pair of *T. erytreae* specific primers was designed from the mitochondrial cytochrome c oxidase subunit I (COI) region. To design them, sequences from the GenBank database (www.ncbi.nlm.nih.gov) were used (Table 1), including: *T. erytreae*, other citrus pest species, other Psylloidea than *T. erytreae*, and some predators present in citrus crops in Spain. Sequences were aligned using ClustalW (www.ebi.ac.uk/Tools/msa/clustalw) and primers were designed as described in Agustí et al. (2003b).

DNA from individual insects was extracted using SpeedTools Tissue DNA Extraction Kit (Biotools, Madrid, Spain), eluted in 100 µl of BBE buffer provided by the manufacturer and stored at -20°C. Negative controls were added to each DNA extraction set. The whole body was used for all insects, except for coccinellids, from which the elytra were removed, and earwigs, from which only the abdomen was used for DNA extraction, as done by Romeu-Dalmau et al. (2012). PCR reaction volumes (20 µl) contained 2 µl of resuspended DNA, 10 µl of Master Mix (Biotools, Madrid, Spain) and 0.4 µl of each primer [10 µM]. Samples were amplified for 35 cycles at 94°C for 30 s, 63°C for 30 s and 72°C for 45 s in a 2720 thermal cycler (Applied Biosystems, Foster

City, CA, USA). A first cycle of denaturation at 94°C for 2 min and a final extension at 72°C for 2 min was carried out. *Trioza erytreae* DNA and water were always included as positive and negative controls, respectively. PCR products were analysed by electrophoresis in 2.4% agarose gels stained with GelRed® (Biotium, Hayward, CA) and visualized under UV light.

2.2. Species specificity

The specificity of the designed primers was tested by attempting to amplify the DNA of other psyllids species, some other citrus pests, some potential predators and one parasitoid of *T. erytreae* liberated in Spain and Portugal as biocontrol agent (Table 1). Three to five individuals of each species were tested, except for *T. erytreae*, which we tested 10 (5 adults and 5 nymphs). To ensure the presence of DNA in specimens that were not amplified with specific primers, we double-checked with a pair of universal arthropod primers (16SLR-J-12961 and 16SLR-N-13398) (Simon et al., 1994). For this amplification, PCR reaction volumes (25 ml) contained 2 ul of DNA template, 0.2 mM of each primer, 1.25 U of Taq DNA polymerase (Invitrogen), 0.2 mM dNTPs (Promega) and 2.5 mM of MgCl2 in the manufacturers' reaction buffer. Samples were amplified for 40 cycles at 95 ºC for 30 s; at 45 ºC for 30 s; and at 72 ºC for 60 s. The first cycle of denaturation was done at 95 ºC for 15 s, and a final extension was done at 72 ºC for 5 min.

2.3. *Prey DNA decay rates*

Chrysoperla carnea and *C. montrouzieri* larvae (2^{nd} instar) feeding trials were carried out to determine the decay rates of *T. erytreae* within their gut. They were conducted in the Canary Institute of Agrarian Research (ICIA) in Tenerife (Canary Islands, Spain), where the pest was present. Nymphs of *T. erytreae* were collected on infested leaves in citrus groves (*Citrus lemon* (L.)) Oskeck) in northern Tenerife and transferred to the laboratory in a portable fridge. Predator larvae were purchased from Koppert© and they were individually placed in 1.5 ml tubes with a cotton soaked in water as a lid and humidity source, where they were in starvation for 48h at controlled conditions of 24°C and 16:8 (L:D) photoperiod. After that, each predator larva was transferred to a plastic container (2.5 cm diameter x 1.5 cm high) with a piece of infested citrus leaf containing 10 nymphs of *T. erytreae* (1^{st}-3^{rd} instar) for 1h at room temperature. After this period, consumed *T. erytreae* nymphs were counted and only those larvae that fed on 2 to 6 nymphs were immediately frozen (t=0) at -20°C or maintained for 4h and 12h, at 24°C and 16:8 (L:D) photoperiod, and then frozen to be analysed. Other predator larvae used as negative controls were previously starved for 48h at the same controlled conditions and immediately frozen without ingestion. Ten individuals were tested for each period of time. Each predator was tested up to 3

141 times and considered positive if *T. erytreae* DNA was detected in one, meaning that if the first
142 PCR was negative, we conducted a second one, and up to third one if the second was negative.
143 This method was conducted to avoid false PCR negatives, as done in Monzó et al. (2010) and
144 Gomez-Polo et al. (2015, 2016). The number of positive predators was recorded, and the
145 percentage of positives was calculated for each post-ingestion period. The time interval associated
146 with 50% positive responses (i.e. detectability half-life) was calculated by reverse prediction from
147 best-fitted equations.

148

149 *2.4. Field sampling and analysis of field-collected predators*

150 Potential predators of *T. erytreae* were collected in three sampling locations (and dates) on
151 infested citrus trees where eggs, larvae and adults of *T. erytreae* were observed. The first sampling
152 was in October 2018 in Tenerife (Canary Islands, Spain), in three small (<1.5 ha) citrus groves
153 (28° 29’ 21.6” N, 16° 21’ 20.3” W; 28° 22’ 42.1” N, 16° 32’ 10.9” W; and 28° 23' 34.6" N, 16°
154 32' 14.6" W). The second sampling was in June 2019 in one lemon grove in Gran Canaria (Canary
155 Islands, Spain) (28° 03' 45.9"N, 15° 34' 28.9"W). The third sampling was in November 2019 on
156 isolated citrus trees located in private gardens or urban areas in Pontevedra (Galicia, NW Spain)
157 (42° 30′ 0″ N, 8° 48′ 0″ W).

158 Collection of these predators was conducted by beating only those flushes observed to have a high
159 infestation of *T. erytreae* to ensure the prey presence was not limited. These flushes were beaten
160 three times on a white tray. Each predator was collected from the tray, placed in a 1.5 ml tube and
161 transferred to the laboratory in a portable fridge. Once in the laboratory tubes were stored at -
162 20°C up to DNA extraction. Each field-collected predator was also tested up to 3 times and
163 considered positive if *T. erytreae* DNA was detected in one of them to avoid false PCR negatives.

164 Before gut content analysis by PCR, predators were morphologically identified using taxonomic
165 keys and bibliographic references (Albouy and Caussanel, 1990; Barrientos, 1988; Eizaguirre,
166 2007; Gómez and Espadaler, 2007; Noualhier, 1893; F. García-Marí, personal comunication).
167 Specimens of the genus *Orius* and hoverfly larvae were identified by molecular methods
168 previously developed (Gomez-Polo et al., 2013 and Gomez-Polo et al., 2014, respectively).

169

170 **3. Results**

171 *3.1. Primer design*

172 A pair of *T. erytreae* specific primers was successfully designed from the COI region, which
173 amplified a fragment of 194 bp. Primer sequences were: 5' GAGGATATTCAGTAGATACTGC

3' (Te2F) and 5' CTGCTAAAACAGGTAATGCC 3' (Te3R). None of the species tested for specificity with this pair of primers was amplified (Table 1), showing their high specificity. When we double-checked with the universal primers, all of them were amplified, indicating the presence of insect DNA in all samples.

3.2. Prey DNA decay rate

PCR analysis of the feeding trials of *C. carnea* larvae, showed a 100 % detection of the tested larvae for *T. erytreae* DNA immediately after feeding (t=0) (Fig. 1). At 4h after feeding, detection decreased to 50%, and dropped to 20% at 12h after ingestion. Feeding trials of *C. montrouzieri*, showed a 70% detection of the tested larvae at t=0, decreasing to 50% at t= 4h, as happened with *C. carnea*, and to 30% at 12h after ingestion. Detection of *T. erytreae* DNA in both predators was better fitted to an exponential decay, with an R^2 value of 0.9879 for *C. carnea*, and 0.9948 for *C. montrouzieri*. Detectability half-life calculated from these equations (Fig. 1) was situated at 4.78h and 4.48h, respectively.

3.3. Field sampling and analysis field-collected predators

A total of 479 potential predators were collected in the three sampling dates (Appendix A), which were all identified to species or genus level, except spiders, which were identified to family level. In some taxa, the number of collected individuals was very low, but they were still analysed in order to better characterize the range of potential predators of *T. erytreae*. In the PCR analysis, 45.7% of them were tested only once, because they were positive at the 1st PCR. Those negative predators were tested a second time, having 12.9% of positives, and 11.5% of them were positives at the 3rd PCR. Therefore, 29.9% of the analysed predators were negative after 3 chances.

Anthocoridae was the most abundant taxon, with the highest collection of individuals (N=202), and with the species *Orius laevigatus* Fieber (Hemiptera: Anthocoridae) as the most abundant (N=168, all collected in Galicia); followed by Coccinellidae (N=84), with *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) as the most abundant species (N=32, all collected in Tenerife) (Appendix A).

The percentage of positive predators for the detection of *T. erytreae* DNA in their gut grouped by family (or order in the case of the spiders) is shown in Fig. 2. In total, 70% of the analysed predators gave a positive detection of *T. erytreae* DNA. A certain percentage of positive individuals was detected in all groups, ranging from 100% in syrphids to 23% in ants (Fig. 2). Also, Hemerobiidae with 94% of positive individuals showed a high detection, followed by other five families: Erythreidae, Anthocoridae, Miridae, Forficulidae and Chrysopidae, which showed

more or around 80% of detection. Araneae and Coccinellidae showed around 50% of detection both.

Considering the sampling location of the collected predators, 311 of them were collected in Galicia and 74.6% were positive for *T. erytreae* DNA. In the Canary Islands, 90 individuals were collected in Gran Canaria, with a 72.2% of positive, and 58 individuals were collected in Tenerife, with 43.1% of positive of *T. erytreae* DNA.

4. Discussion

The pair of primers designed to detect *T. erytreae* in predator gut contents was specific enough to detect the target species avoiding the detection of other pests, predators and even a parasitoid potentially present in citrus crops. This validates the potential use of these molecular markers for monitoring interactions between *T. erytreae* and some predator species. On the other hand, the amplified COI fragment is the same for all *T. erytreae* developmental stages, as it happens in all DNA-based predation studies, meaning that is not possible to know whether a predator had been feeding on eggs, nymphs or adults. Because many of the tested predators might feed on eggs, it would be interesting to conduct laboratory feeding trials of *T. erytreae* eggs in further studies and to calculate the half-life detection of this developmental stage. In the present study, we have shown that the half-life detection of 1st-3rd instar larvae of this psyllid by two generalist predators showed that *T. erytreae* detection is possible in 50% of the cases up to 4.5h under the conditions tested, then showing the most recent feeding episodes. This half-life detection time is similar to the Asian citrus psyllid *D. citri* DNA half-life detection ingested by the lacewing *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) and the ladybird *Hippodamia convergens* Guérin-Menéville (Coleoptera: Coccinellidae) obtained by Nanini et al. (2019), which were 5.5 h and 6.1h, respectively.

Our field sampling study demonstrated that a wide range of generalist predators include *T. erytreae* in their diets, being *O. laevigatus* the most abundant predator collected in Galicia, the highest consumer of *T. erytreae* (82% of them). We have also demonstrated that other anthocorid species ingested *T. erytreae* (Appendix A). Among them, *O. laevigatus* and *Anthocoris* sp. have been occasionally observed feeding on *T. erytreae* in citrus groves in the Canary Islands (Estévez et al., 2018). It is important to note that almost all the analysed anthocorids were collected in Galicia, where they were found in isolated citrus trees in gardens, instead of in citrus orchards. In the Mediterranean basin, *Orius* spp. are not frequently found in citrus groves, but the fact that they were detected with this abundance on those citrus trees make them potential candidates for the biological control of this psyllid species. In South Africa, non-identified anthocorid individuals were also observed feeding on *T. erytreae* nymphs (van den Berg et al., 1987).

Coccinellidae was the family with the highest number of species collected and analysed (Appendix A). Even though only one specimen was collected in most of these species, only in five of them *T. erytreae* DNA was not detected. *Harmonia axyridis* was the most abundant coccinellid in one of the citrus groves sampled in Tenerife, and 40% of them were positive for *T. erytreae* DNA. This coccinellid species had been observed to feed on eggs and nymphs of *T. erytreae* in laboratory experiments (Estévez et al., 2018), as well as to feed on *D. citri* in Florida and Brazil (Michaud and Olsen, 2004; Monzó et al. 2014; Nanini et al. 2019). The second coccinellid regarding abundance was *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae), showing also ingestion of *T. erytreae* in 76% of the cases, which could also be interesting for IPM programs in Spain, because is very common in Mediterranean citrus agroecosystems (Boukhris-Bouhachem, 2011; Kavallieratos et al., 2004). Khan et al. (2016) have described this coccinellid as good commercial predator for *D. citri* nymphs. Other analysed coccinellids, like *C. montrouzieri*, *Rodolia cardinalis* (Mulsant), *Rhyzobious* spp. and *Coccinella* spp., in which we have detected the target psyllid with different percentages (Appendix A), had been also found in citrus groves in Valencia (Spain) (Alvis, 2003). It is well known that some of them have an important role reducing populations of various citrus key pests (Jacas and Urbaneja, 2010).

Predation of *T. erytreae* by Neuroptera was previously described in citrus groves in South Africa (van den Berg et al., 1987), and in the case of *C. carnea* was also frequently observed in citrus groves in Tenerife (Estévez et al., 2018). The present study confirmed the consumption of *T. erytreae* by *C. carnea*, as well as by the brown lacewing *Hemerobius eatoni* Morton (Neuroptera: Hemerobiidae), an endemic species of the Canary Islands. The mirid bug *Aetorhinella parviceps* Noualhier (Hemiptera: Miridae) was another endemic species of the Canary Islands which has also been recorded predating *T. erytreae* in the present study. More than 80% of the analysed individuals of both endemic species were positive for the target DNA, demonstrating how two native predators were able to feed on a new invasive species as *T. erytreae*.

All analysed syrphid larvae were positive for the target DNA, make them also potential candidates for the biological control of *T. erytreae*. The molecular method used to identify the syrphid species allowed the identification of only ten of them (10 specimens of *Meliscaeva auricollis* (Meigen) (Diptera: Syrphidae)). The remaining six larvae were not identified with the method used, indicating that they might be other syrphid species than those identified with this multiplex PCR.

The earwig *F. auricularia* was collected in Galicia as nymphs and adults, and most of them (82%) fed on *T. erytreae*. This species was also recorded as predator in citrus orchards by Romeu-Dalmau et al. (2012), since the DNA of the main citrus aphids was detected in their gut. Although earwigs could have a potencial predator role in citrus orchads, it remains a controversial subject,

277 particullarly in young trees, where earwigs can cause damages due to their phytophagus behaviour
278 (Grafton-Cardwell et al., 2003; Kallsen, 2006).

279 Regarding Araneae, individuals of nine families were analysed, and five of these families fed on
280 *T. erytreae* (Appendix A). In Spain, some studies emphasize the abundance of these predators in
281 citrus groves and their relationship with some pests, like aphids, the mussel scale *Lepidosaphes*
282 *beckii* (Newman) (Hemiptera: Diaspididae), and the Mediterranean fruit fly *Ceratitis capitata*
283 (Wiedemann) (Diptera: Tephritidae) (Alvis, 2003; Monzó et al., 2010). In South Africa they have
284 been cited to contribute in the reduction of *T. erytreae* populations, mostly of species belonging
285 to Salticidae (van den Berg et al., 1992). For *D. citri*, spiders are reported as predominant predator
286 group on *D. citri* colonies (Qureshi and Stansly, 2009), and the families Anyphaenidae and
287 Salticidae have been reported feeding on this psyllid in Florida (Michaud, 2002), as it happens in
288 the present study for *T. erytreae*.

289 As cited by Estévez et al. (2018), we observed larvae of *Leptus* spp. parasitizing adults of *T.*
290 *erytreae* in the field. The analysis of some adults of these erythraeid mites showed a 90% of adults
291 positive for the target DNA. Therefore, they could contribute to the biological control of *T.*
292 *erytreae*.

293 Two ant species were also analysed for the presence of the target DNA in their gut, *Lasius grandis*
294 Forel and *Linephitema humile* (Mayr) (Hymenoptera: Formicidae), showing the lowest
295 percentage of positive detection of *T. erytreae* DNA (Appendix A). The most frequent species in
296 citrus trees in Spain is *L. grandis*, that together with *L. humile*, were the most abundant ants in the
297 sampled citrus trees. Both species feed mainly on carbohydrates and have been reported attending
298 several honeydew-producing hemipterans in citrus crops (Calabuig et al., 2014; Martínez-Ferrer
299 et al., 2003; Martínez-Ferrer and Campos-Rivela, 2017; Pekas et al., 2011; Zina, 2008). However,
300 both ant species are omnivorous. Predation on honeydew-producing hemipterans by ants has been
301 reported for some species, depending on their population density and the availability of honeydew
302 (Billick et al., 2007; Sakata, 1994). The detection of *T. erytreae* in their gut revealed that both
303 analysed ant species fed on the target insect, and therefore, its predatory role in the biological
304 control of this pest should not be underestimated. Even if ingestion has not been detected in some
305 of them, sometimes ants transport the prey to the nest without having ingested them (Cerdà and
306 Dejean, 2011).

307 In summary, the pair of primers designed in this study and the PCR method developed allowed
308 the detection of *T. erytreae* in the gut content of field collected predators in citrus trees with tender
309 flushes infested by the psyllid. Some of these generalist predator species are not common in citrus-
310 growing areas, such as *O. laevigatus.* Since this species is commercially available, its inundative
311 release could be considered in some particular cases. Most of them are commonly present in citrus

agroecosystems of the Mediterranean coast of Spain, which means that they might contribute to the biological control of this citrus pest if it will arrive to this area.

CRediT authorship contribution statement

Paula Molina: Methodology, Investigation, Writing-Original draft preparation, review and editing. **María Teresa Martínez-Ferrer:** Conceptualization, Writing-Review and editing, Project administration. **José Miguel Campos-Rivela:** Conceptualization, Writing-Review and editing. **Jordi Riudavets:** Conceptualization, Writing-Review and editing. **Nuria Agustí:** Conceptualization, Methodology, Supervision, Writing-Original draft preparation, review and editing.

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Table 1. Species used for primer design (GenBank accession number indicated), and species tested for specificity of the *T. erytreae*-specific primer pair Te2F/Te3R, as well as with the universal pair of primers 16SLR-J-12961/16SLR-N-13398. Also indicated the origin of the samples, all in Spain. NA= not applicable.

				Primer design	Specificity test		
Group	Order	Family	Species	GenBank accession number	Origin	Te2F/Te3R PCR detection	16SLR-J-12961 /16SLR-N-13398 PCR detection
Citrus pests	Hemiptera	Triozidae	*Trioza erytreae* Del Guercio	KU517195 KY754656 KY754588 KY754594	Tenerife	+	+
			Trioza urticae (L.)	KY011195	Barcelona	-	+
			Lauritrioza alacris (Flor)	MG988839	Barcelona	-	+
		Aleyrodidae	*Aleurothrixus floccosus* (Maskell)	KF059956	Tenerife	-	+
		Aphidini	*Aphis gossypii* Glover	EU930154	Tarragona	-	+
			Aphis spiraecola Patch	JX844415	Tarragona	-	+
		Diaspididae	*Aeonidiella aurantii* (Maskell)	HM474070		NA	NA
		Coccidae	*Saissetia coffeae* (Walker)	NA	Tenerife	-	+
		Monophlebidae	*Icerya purchasi* Maskell	NA	Tarragona	-	+
		Pseudococcidae	*Planococcus citri* (Riso)	JQ085543		NA	NA
	Lepidoptera	Gracillariidae	*Phyllocnistis citrella* Stainton	KF492017	Tarragona	-	+
Other Psylloidea	Hemiptera	Homotomidae	*Macrohomotoma gladiata* Kuwayama	MG988795		NA	NA
		Psyllidae	*Cacopsylla alaterni* (Foerster)	AY100431		NA	NA
			Euphyllura olivina Costa	KR052011		NA	NA
			Psyllopsis fraxinicola Foerster	KU517186		NA	NA
Predators	Hemiptera	Anthocoridae	*Orius laevigatus* Fieber	NA	Lleida	-	+
			Orius majusculus (Reuter)	NA	Lleida	-	+
			Orius niger Wolff	NA	Lleida	-	+
	Coleoptera	Coccinellidae	*Cryptolaemus montrouzieri* Mulsant	FM210142	commercial	-	+
	Neuroptera	Chrysopidae	*Chrysoperla carnea* (Stephens)	AY743793	commercial	-	+
Parasitoid	Hymenoptera	Eulophidae	*Tamarixia dryi* (Waterston)	NA	Tenerife	-	+

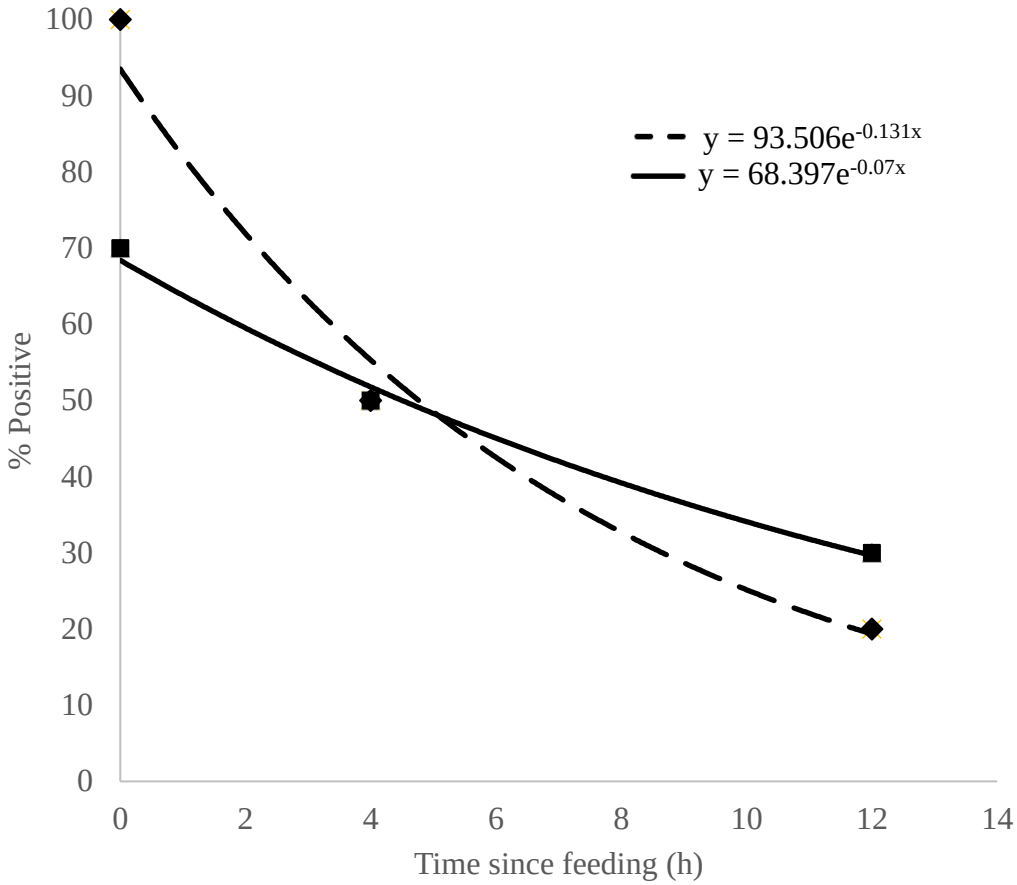
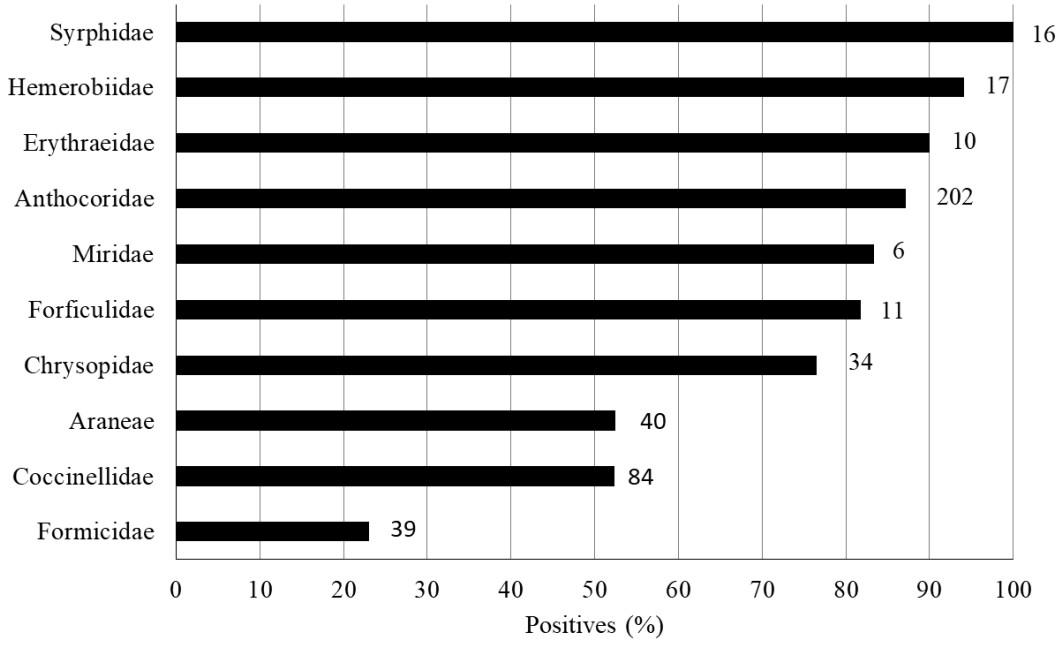


Figure 1. Detection of *Trioza erytreae* DNA ingested by *Chrysoperla carnea* (♦, discontinued line) and *Cryptolaemus montrouzieri* (■, continued line) larvae at different times after ingestion.



501

502 **Figure 2.** Percentage of PCR positive detection using the *T. erytreae*-specific primers showed by
503 family, except for the order Araneae. The number at the end of the bar indicates the number of
504 individuals tested per taxa.

Appendix A. Number of field-collected analysed individuals (N) and percentage of PCR detection (Detection (%)) of each arthropod taxa. The location of collection in Spain and the developmental stage are also indicated. NI = non identified.

Order	Family	Genus/ Species	Stage	Location	N	Detection (%)
Araneae	Anyphaenidae		adult	Galicia	3	100
	Araneidae		adult	Tenerife	3	100
				Gran Canaria	1	0
				Galicia	15	46.7
	Dictynidae		adult	Gran Canaria	1	100
	Linyphiidae		adult	Tenerife	1	0
				Gran Canaria	4	0
	Mimetidae		adult	Tenerife	1	0
	Philodromidae		adult	Galicia	1	0
	Salticidae		adult	Tenerife	3	66.7
				Gran Canaria	4	100
	Theridiidae		adult	Tenerife	1	0
	Thomisidae		adult	Gran Canaria	1	0
				Galicia	1	100
Coleoptera	Coccinellidae	*Adalia bipunctata* (L.)	adult	Galicia	17	76.5
		Adalia decempunctata (L.)	adult	Galicia	1	100
		Clitostethus arcuatus (Rossi)	adult	Galicia	4	0
		Coccinella miranda Wollaston	adult	Gran Canaria	1	100
		Coccinella septempunctata L.	adult	Gran Canaria	1	100
				Galicia	1	100
		Cryptolaemus montrouzieri Mulsant	larva	Gran Canaria	2	100
			adult	Gran Canaria	4	100
		Exochomus quadripustulatus (L.)	adult	Galicia	1	100
		Harmonia axyridis (Pallas)	adult	Tenerife	52	40.6

		Hippodamia variegata (Goeze)	adult	Gran Canaria	1	0
		Propylea quatuordecimpunctata (L.)	adult	Galicia	1	100
		Rhyzobious chrysomeloides (Herbst)	adult	Galicia	1	100
		Rhyzobious forestieri (Mulsant)	adult	Galicia	7	42.9
		Rhyzobious litura (Fabricius)	adult	Gran Canaria	1	0
		Rodolia cardinalis (Mulsant)	adult	Tenerife	1	0
				Galicia	5	20
		Scymnus canariensis Wollaston	adult	Gran Canaria	1	0
		Scymnus rubromaculatus (Goeze)	adult	Gran Canaria	1	100
		Stethorus punctillum Weise	adult	Gran Canaria	1	0
Dermaptera	Forficulidae	*Forficula auricularia* L.	nymph	Galicia	7	71.4
			adult	Galicia	4	100
Diptera	Syrphidae	NI	larva	Galicia	6	100
		Meliscaeva auricollis (Meigen)	larva	Galicia	10	100
Hemiptera	Anthocoridae	*Anthocoris* sp	nymph	Galicia	3	100
			adult	Galicia	18	83.3
		Orius albidipenis Reuter	adult	Galicia	1	100
		Orius laevigatus Fieber	adult	Galicia	168	82.1
		Orius majusculus Reuter	adult	Galicia	7	85.7
		Orius niger Wolf	adult	Galicia	1	100
		Orius spp.	adult	Tenerife	3	66.7
				Galicia	1	100
	Miridae	*Aetorhinella parviceps* Noualhier	adult	Gran Canaria	6	83.3
Himenoptera	Formicidae	*Lasius grandis* Forel	adult	Galicia	14	21.4
		Linepithema humile (Mayr)	adult	Gran Canaria	19	31.6
				Galicia	6	0
Neuroptera	Chrysopidae	*Chrysoperla carnea* (Stephens)	larva	Tenerife	13	38.5
				Gran Canaria	20	100

				Galícia	1	100
	Hemerobiidae	*Hemerobius eatoni* Morton	larva	Gran Canaria	6	83.3
			adult	Gran Canaria	11	100
Trombidiformes	Erythreidae	*Leptus* spp	adult	Gran Canaria	4	100
				Galicia	6	83.3
				Total	479	68.7