Preventing HLB epidemics for ensuring citrus survival in Europe

[D3.1] Report about new sampling methods for adults and nymphs of T. erytreae in ornamental citrus trees and orchards. (IPB, PU, M12)

Deliverable No.	D3.1	Work Package No.	WP3	Task/s No.	3.1
Work Package Title	BIOECOLOGY OF TRIOZA ERYTREAE AND POTENTIAL TO SPREAD				
Linked Task/s Title	Task 3.1. Development of a sampling methodology to assist and optimize the monitoring and containment of the spread of T. erytreae in Europe				
Status	Final		(Draft/Draft Final/Final)		
Dissemination level	PUBLIC		(PU-Public, PP, RE-Restricted, CO-Confidential)		
Due date deliverable	31-05-2020 (12 months)		Submission date		19-10-2020
Deliverable version	PreHLB.eu				

Horizon 2020
European Union Funding
for Research & Innovation

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 817526

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1 EXECUTIVE SUMMARY

The population of *Trioza erytreae* adults was monitored using different sampling methods for comparison in 3 lemon groves located close to the Porto region (Portugal) : (1) Standard yellow sticky traps, (2) Saturn yellow sticky traps, (3) B-vac sampling, (4) Sweep net, and (5) Horizontal green tile water trap (Irwin trap). The information was completed through direct observation of damaged leaves and estimation of the number of eggs and nymphs.

The results indicate that the population dynamics of *T. erytreae* show one main peak density at the beginning of August according to the yellow sticky trap catches. The population started to drop gradually in September reaching very low densities in October and remained close to 0 until the beginning of April. The peak density according to B-Vac and sweep net sampling was mid-July but the mean number of catches was much lower than with the yellow sticky traps. The horizontal tile trap caught a low number of individuals but the trap was placed too late, in late September when the *T. erytreae* population was already low. The side and location of the yellow sticky traps had a significant effect but the trap type (either standard or fluorescent Saturn) had no effect on the number of catches. In summary, the standard yellow sticky trap was the best method to monitor the population dynamics of the African citrus psyllid in lemon orchards and a minimum of 3 traps/ha should be used to estimate the mean density of *T. erytreae* with 90% of accuracy.

2 INTRODUCTION

Previous studies have shown that yellow sticky traps could be the best way strategy to monitor *T. erytreae* but no other sampling methods have been tested and compared between each other. Visual inspection has been used in South Africa (van der Kooij et al. 1986), as well as yellow sticky traps placed in citrus orchards (Samways et al. 1986; van den Berg et al. 1991).

The aim of this task was to compare several sampling methods and protocols in the same orchard and location to determine the presence, and decide which strategy would be the best to estimate *T. erytreae* population dynamics in citrus orchards.

3 METHODOLOGY

The population of *T. erytreae* adults was monitored in the same lemon orchard using five different sampling methods for comparison: (1) Standard yellow sticky traps, (2) Saturn yellow sticky traps, (3) B-(blower) - vac, based on leaf blowers for suction sampling, (4) entomological sweep net, and (5) Horizontal green tile water trap (Irwin trap). The information was completed through direct observation of damaged leaves and estimation of the number of eggs and nymphs. Three lemon groves at different locations close to the Porto region (Portugal) were selected for our surveys: Caracoi, Caracoi 1, and Vairão. Sampling was conducted from March 2019 to April 2020 on a weekly basis for the case of the sticky traps and Irwin trap, and on a fortnightly basis in the case of B-Vac and sweep net sampling.

Estimation of *T. erytreae* adult densities: A total of 10 sticky traps (5 standard and 5 saturn, yellow trap) each one with a sticky area of 25 × 20 cm were installed in each lemon grove. The traps were uniformly distributed throughout the grove and replaced on a weekly basis to be cleaned and re-glued. The number of adults of *T. erytreae* captured was counted. Counts of individuals were done on both sides of the traps. Traps were aligned with the plantation row so that each side faced two consecutive inter-rows. Side “A” was North-West oriented whereas side “B” was South-East oriented.

A single Irwin trap was placed in a central position in Caracoi at the canopy level. The trap was filled up with water plus ethylene-glycol at 50% and a second container was placed below to avoid losing samples because of rain. The captured individuals were collected weekly, filtered, and preserved in ethanol 70%.

The B-Vac sampling was conducted on a fortnightly basis at 10 randomly selected trees per grove. Two samples were collected in each tree canopy by aspiration of tree shoots at the opposite side of each tree canopy (sides A and B). Each B-Vac suction sample lasted 30 seconds. This method was used in Caracoi and Caracoi 1 lemon orchards.

The sweeping sampling was conducted on a fortnightly basis using an entomological sweeping net at the canopy level in 10 randomly selected trees per grove. Each sample consisted of four sweeps around the tree.

The effect of the type of trap (Standard and fluorescent Saturn), the trap side (A and B), and the trap position throughout the grove on the number of captured adults of *T. erytreae* was analyzed by developing a generalized linear mixed model (GLMM) using the abundance data as response whereas type of trap, side, and position were used as drivers. Grove was considered as a random term and since the response corresponded to count data, the Poisson distribution was used. Statistical significance among level factors was assessed using a post-hoc Tukey test ($\alpha = 0.05$).

Estimation of eggs and nymph densities: For this purpose, 10 trees showing symptoms of attack were randomly selected weekly. A shoot was selected in each tree at each cardinal point (i.e. four shoots per tree). At each shoot two types of leaves were selected, the first type consisting of a leaf from 0 to 3 cm in length and the second type consisting of a leaf longer than 3 cm. For each leaf, the number of eggs laid by *T. erytreae*, the number of nymphs, and their development stage were registered. Data was coded as follows:

Presence/absence of shoots - **ND**: No shoot; **0**: Empty shoot.
Abundance of eggs and nymphs - **1**: 1-10 eggs or nymphs; **2**: 11-20 eggs or nymphs; **3**: >20 eggs or nymphs.

Estimation of the minimum sampling effort: The minimum number of sticky traps required to obtain a reliable assessment of the population of *T. erytreae* was estimated using the Taylor´s law (Taylor & Woiwod, 1980) and then solving the Green´s model (Green, 1970).
The Taylor's power law states the relationship between the variance in population size and its mean density as (after linearization):

$\text{Log}(S^2) = \text{Log}\,\alpha + \beta\,\text{Log}(\overline{X})$

where α is the intercept, β is the slope of the regression line, S^2 is the variance, and $\overline{X}$ is the mean.
The minimum number of samples necessary for estimating the mean at a given precision is determined by the Green's model (Green, 1970):

$N = (\alpha + \overline{X}^{\beta-2}) / D^2$

where n is the number of samples, D the precision level, and α and β are the coefficients obtained from the Taylor's power law. The precision level was established at 90% (i.e. D = 0.1). The minimum sampling effort was calculated as the minimum number of traps per hectare.

4 RESULTS

The main results of our sampling methods in the three lemon groves are shown in figures 1 to 5. The population dynamics of *T. erytreae* showed one main peak density at early August in the three orchards surveyed according to the yellow trap catches. The population started to drop gradually in September reaching very low densities in October and remained close to 0 until the beginning of April (Figure 1). The peak density according to B-Vac and sweep net sampling was mid-July but the mean number of catches was much lower than with the yellow sticky traps (Figures 2 and 3). The horizontal tile trap caught a low number of individuals but the trap was placed too late, in late September when the *T. erytreae* population was already low (Figure 4). The side and location of the yellow sticky traps (Figures 5 & 6) had a significant effect but the trap type (either Standard or fluorescent Saturn) had no effect on the number of catches.

Side "A" of the yellow sticky traps captured a statistically significantly higher number of individuals than the opposite side "B" (χ^2 = 548.47; df = 1; P < 0.001) (Figure 7A). Furthermore, the trap position within the grove had a significant effect on the number of captured individuals resulting in a border effect where surrounding traps (positions 1 and 5) captured significantly more individuals than the central ones (χ^2 = 8422.33; df = 4; P < 0.001), (Figure 7B). On the contrary, the trap type (standard yellow or fluorescent Saturn yellow) did not significantly affect the number of captured individuals (χ^2 = 0.98; df = 1; P = 0.32) (Figure 7C).

The number of eggs and nymphs on lemon leaves were highest during the summer months (July and August) (Figures 8 to 11).

In summary, according to our results, the standard yellow sticky trap provides the highest number of catches and is the most time-effective method to sample populations of *T. erytreae* in lemon orchards.

The estimated parameters of the Taylor´s law were α = 0.42 and β = 1.75 (R^2 = 0.96) (Figure 12). Regardless of the type of sticky trap, the minimum sampling effort estimated for a precision level (D) of 0.1 was a mean of three sticky traps per hectare (i.e. six sides of trap) during the outbreak of *T. erytreae* (Figure 13).

5 FIGURES

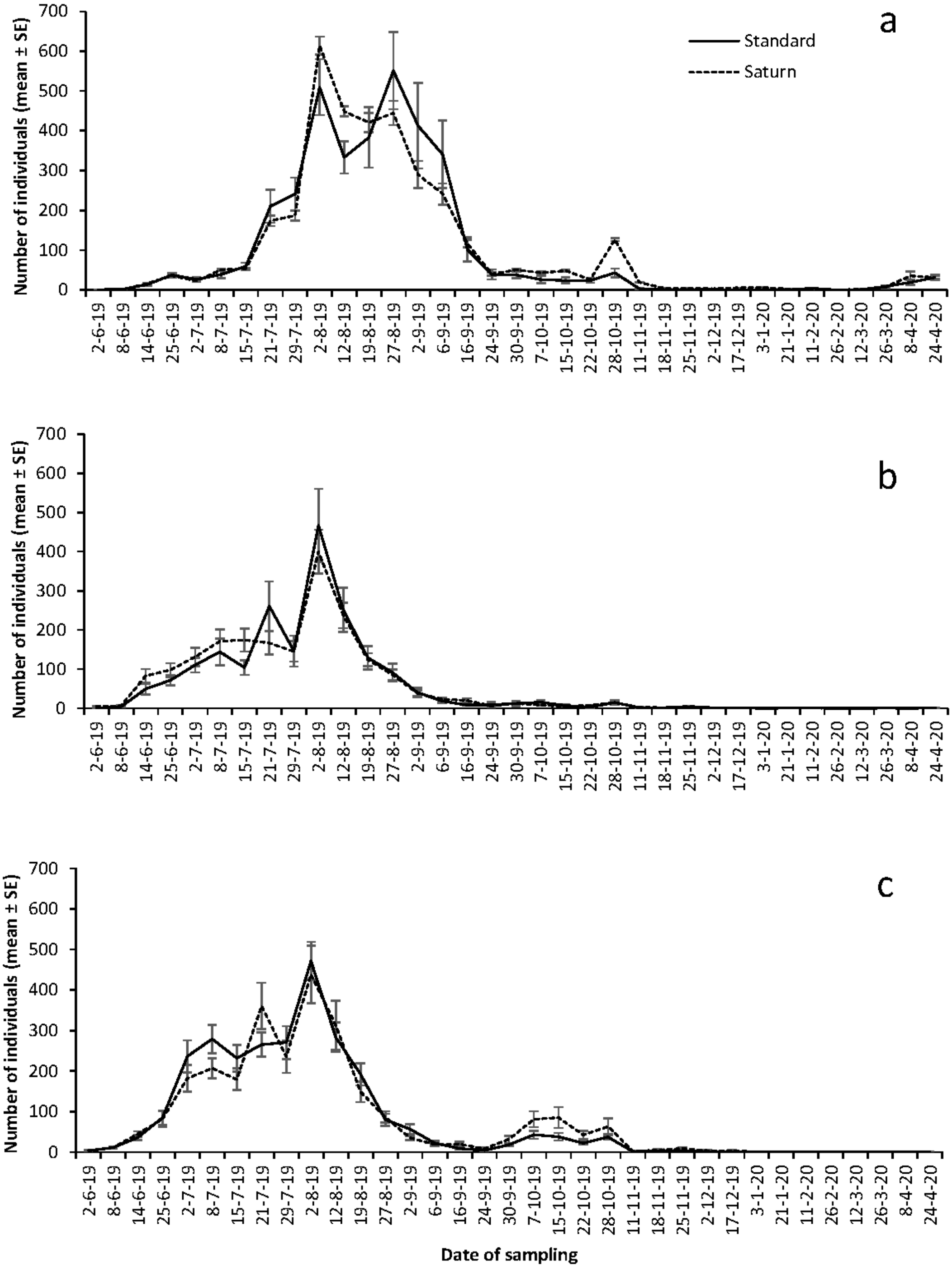

Figure 1. Mean number (± SE) of adults of *Trioza erytreae* captured using standard yellow sticky traps and saturn yellow sticky traps in Vairão (A), Caracoi (B) e Caracoi 1 (C).

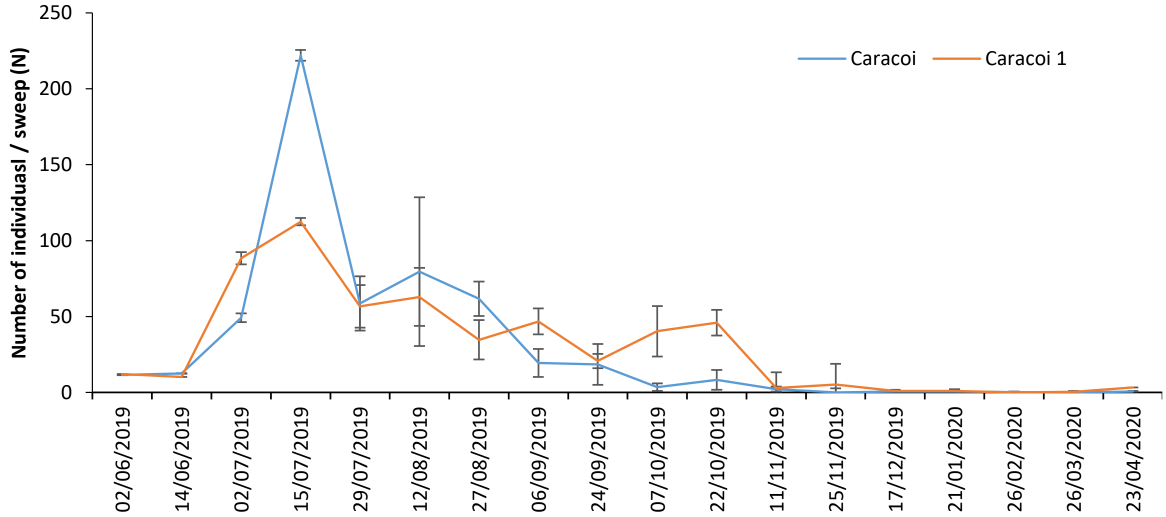

Figure 2. Mean number (± SE) of adults of *Trioza erytreae* captured by sweeping in Caracoi and Caracoi 1.

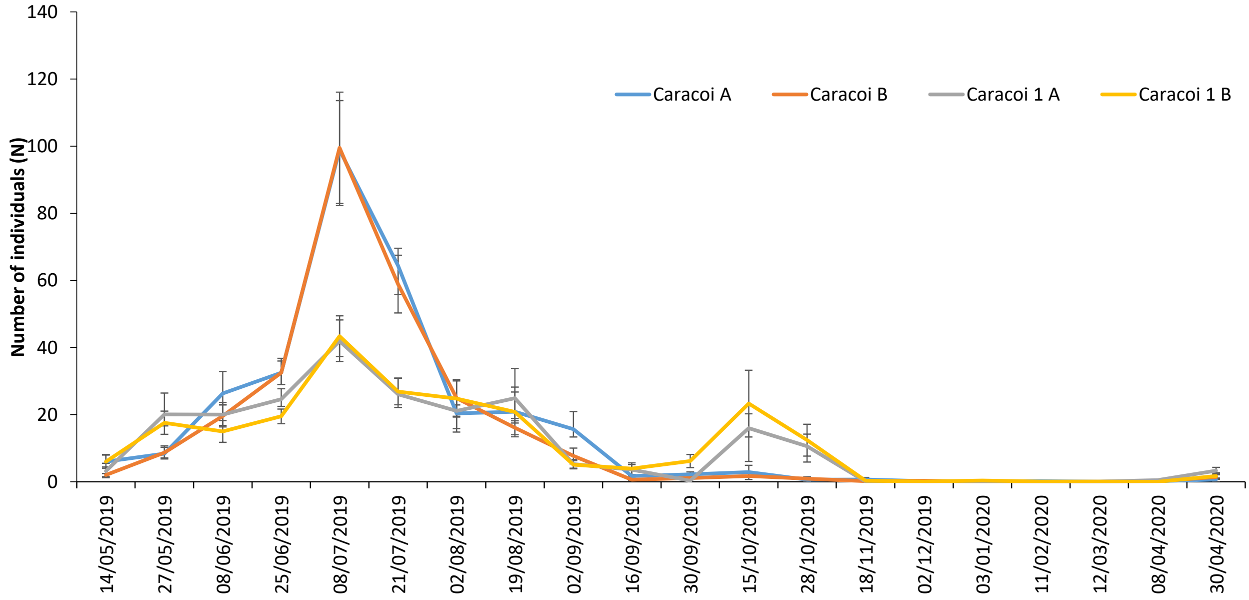

Figure 3. Mean number (± SE) of adults of *Trioza erytreae* captured by B-Vac in Caracoi and Caracoi 1. A and B represent sides of a trap each one facing two consecutive plantation inter-rows.

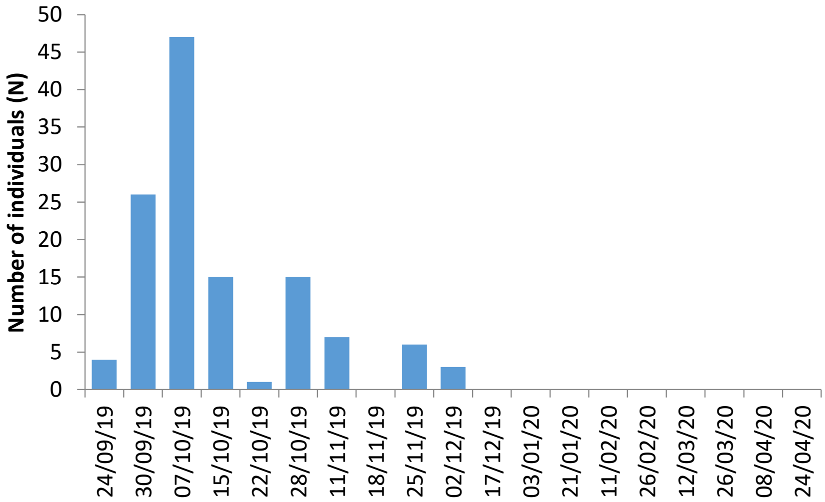

Figure 4. Number of adults of *Trioza erytreae* captured by the Irwin trap in Caracoi. Note that sampling started in late September when the *T. erytreae* population started to drop.

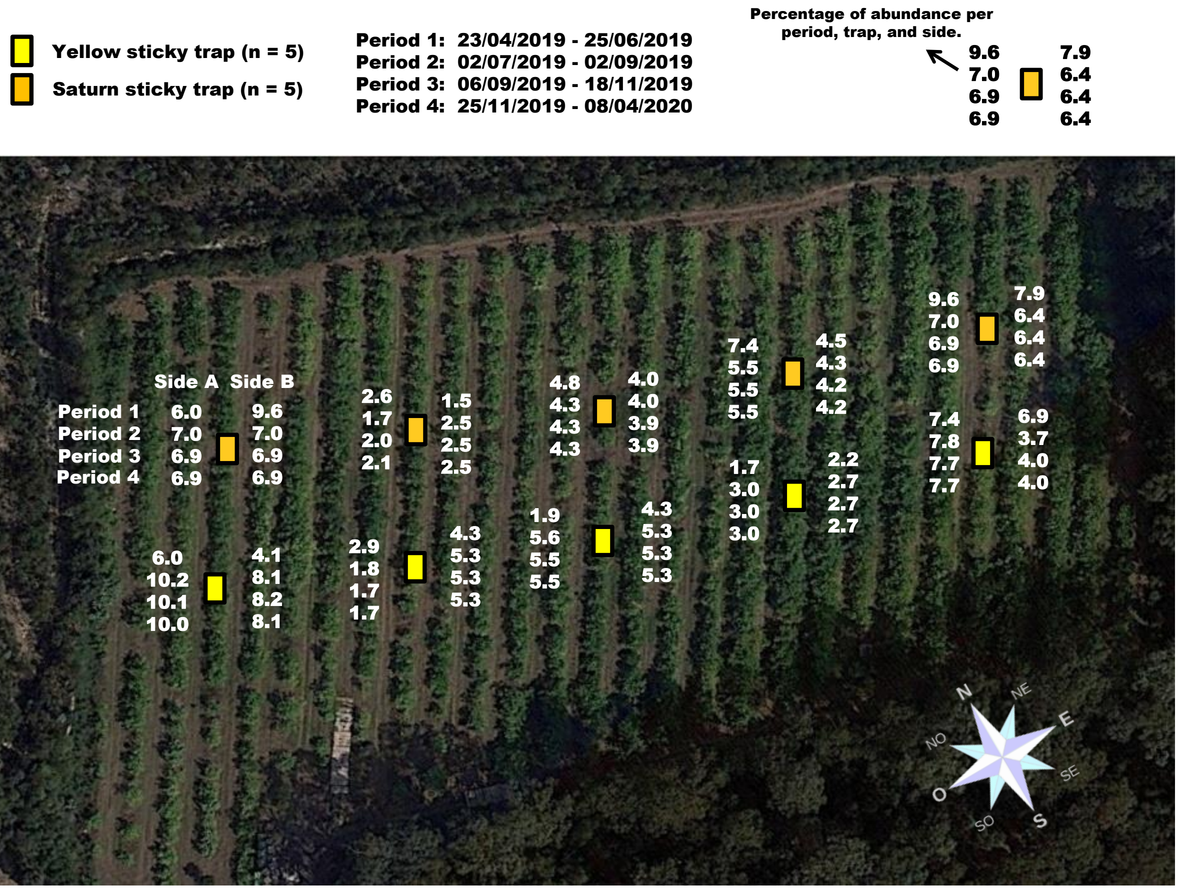

Figure 5. Location of sticky traps and percentage of adult individuals of *Trioza erytreae* captured by trap type, trap side, and trap position in Caracoi pooled for four sampling periods.

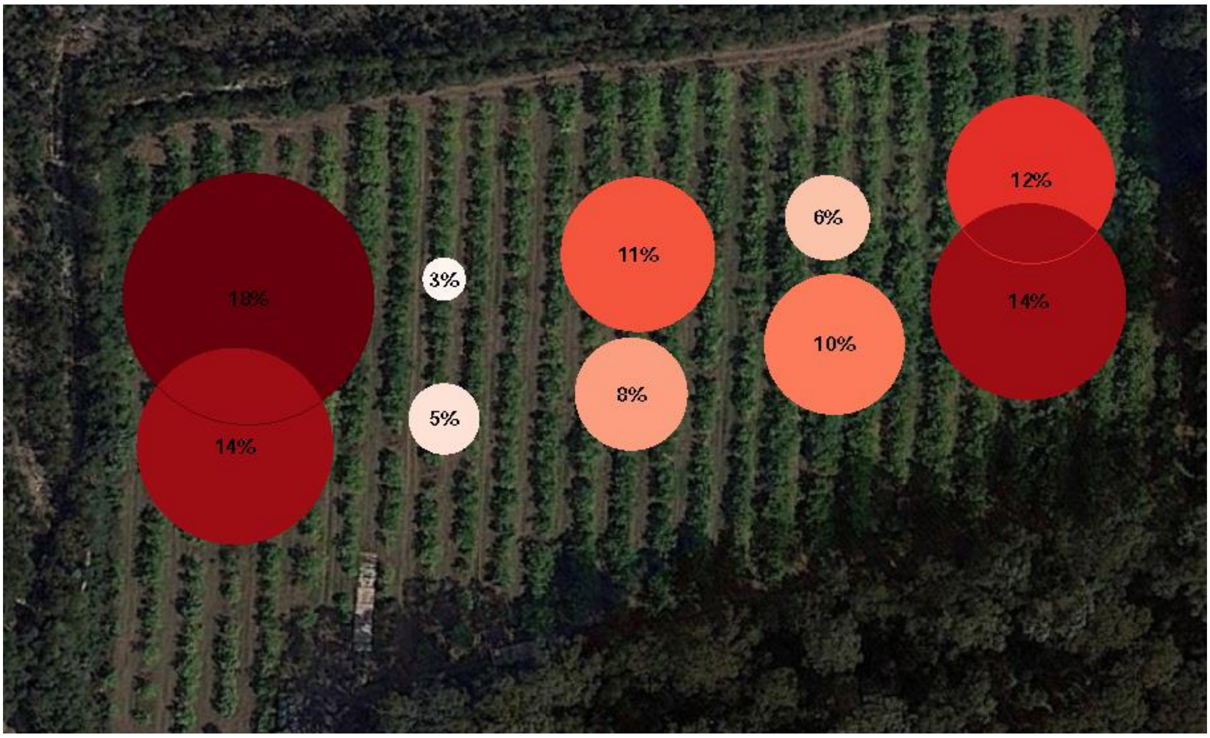

Figure 6. Spatial representation of the distribution of the adult individuals of *Trioza erytreae* captured using sticky traps in Caracoi (see Figure 5). Data was pooled for the whole sampling period. Size and color of circles are proportional to the percentage of captures.

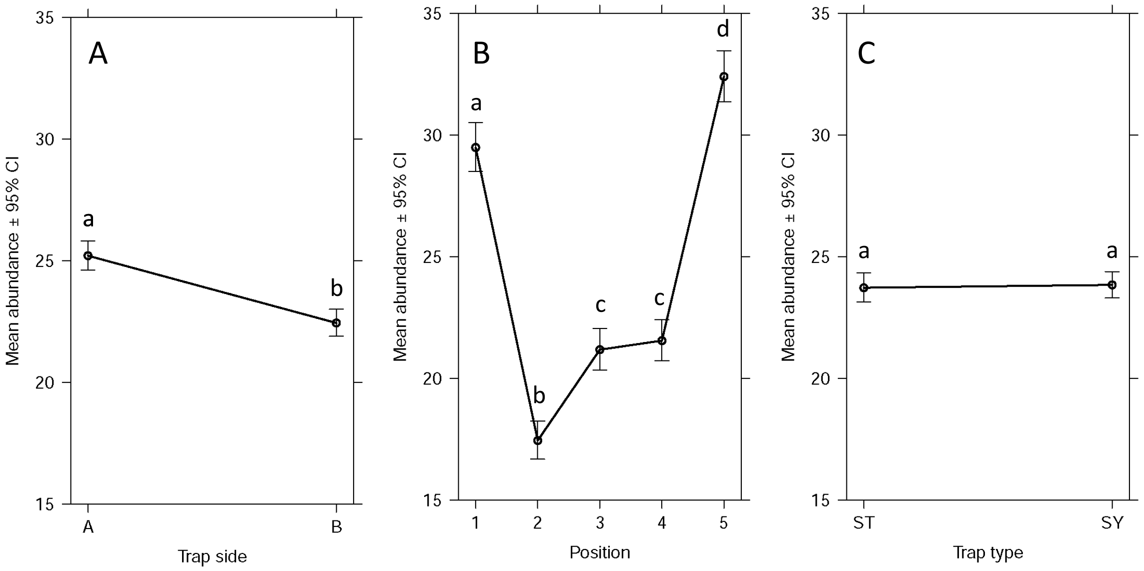

Figure 7. Effect of trap side (A), trap position (B), and type of trap (C) on the number of captured adults of *Trioza erytreae*. Different letters above bars indicate significant differences.

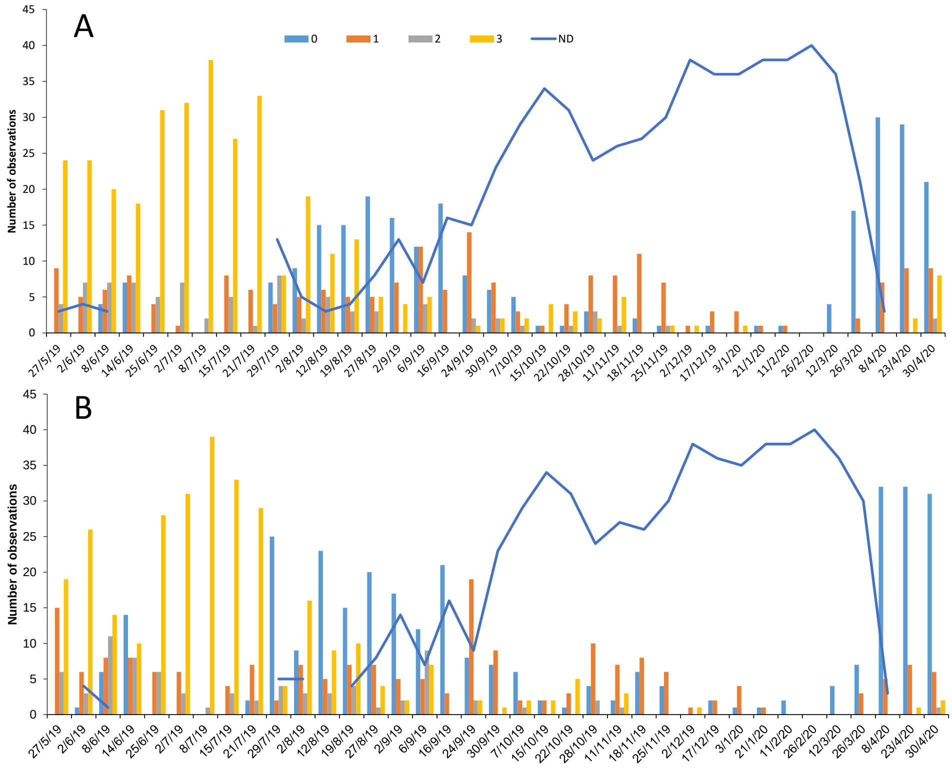

Figure 8. Evolution of the abundance of eggs of *Trioza erytreae* in Caracoi in shoots from 0-3 cm (A) and longer than 3 cm (B). ND: Shoot not available 0: Empty shoot; 1: 1-10 eggs or nymphs; 2: 11-20 eggs or nymphs; 3: >20 eggs or nymphs.

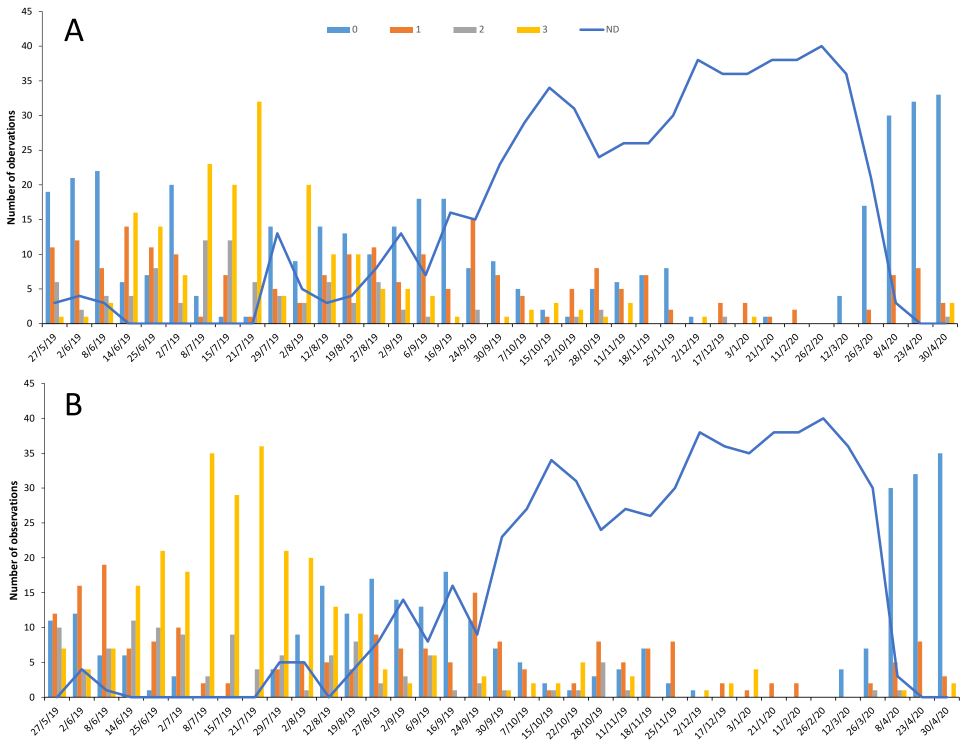

Figure 9. Evolution of the abundance of nymphs of *Trioza erytreae* in Caracoi in shoot from 0-3 cm (A) and longer than 3 cm (B). ND: Shoot not available; 0: Empty shoot; 1: 1-10 eggs or nymphs; 2: 11-20 eggs or nymphs; 3: >20 eggs or nymphs.

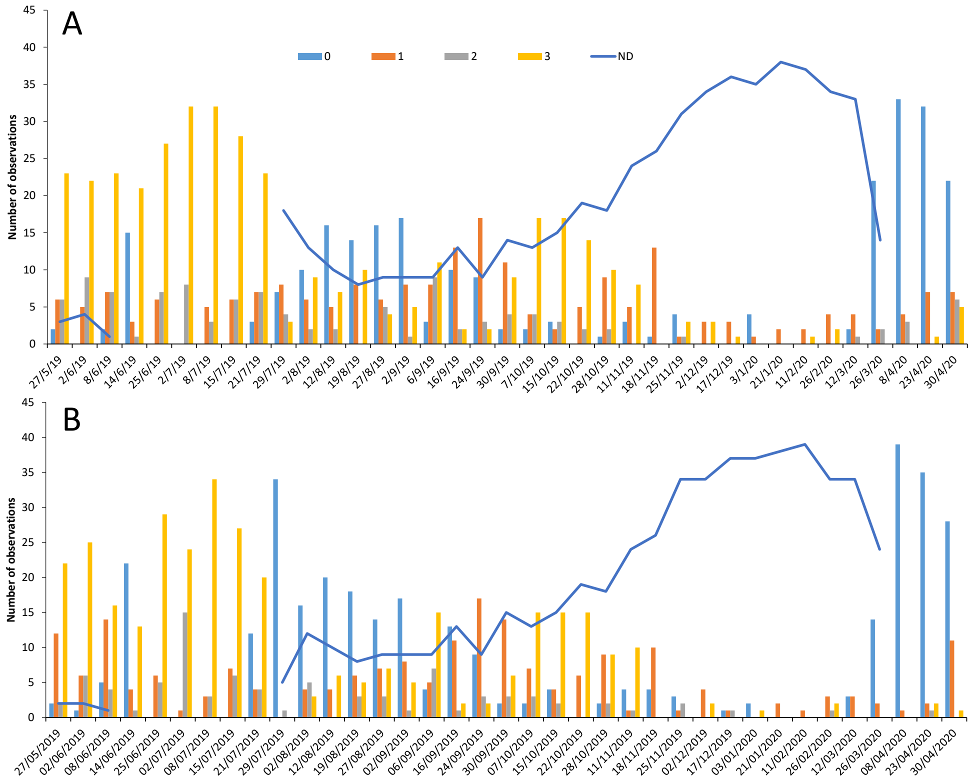

Figure 10. Evolution of the abundance of eggs of *Trioza erytreae* in Caracoi 1 in sprouts from 0-3 cm (A) and longer than 3 cm (B). ND: Shoot not available; 0: Empty shoot; 1: 1-10 eggs or nymphs; 2: 11-20 eggs or nymphs; 3: >20 eggs or nymphs.

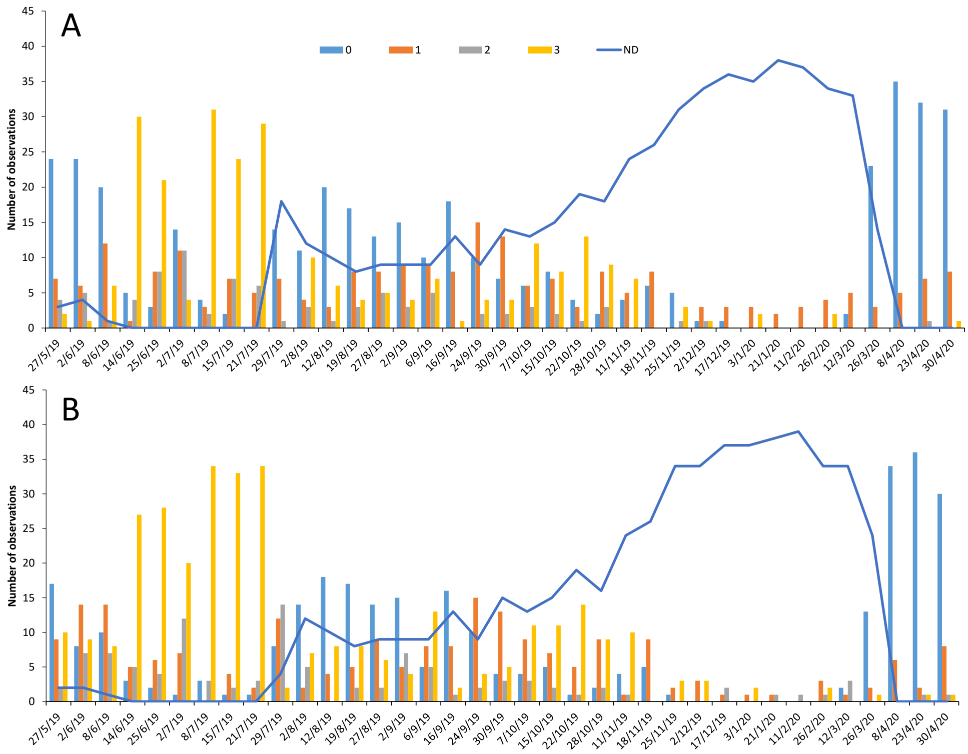

Figure 11. Evolution of the abundance of nymphs of *Trioza erytreae* in Caracoi 1 in sprouts from 0-3 cm (A) and longer than 3 cm (B). ND: Shoot not available 0: Empty shoot; 1: 1-10 eggs or nymphs; 2: 11-20 eggs or nymphs; 3: >20 eggs or nymphs.

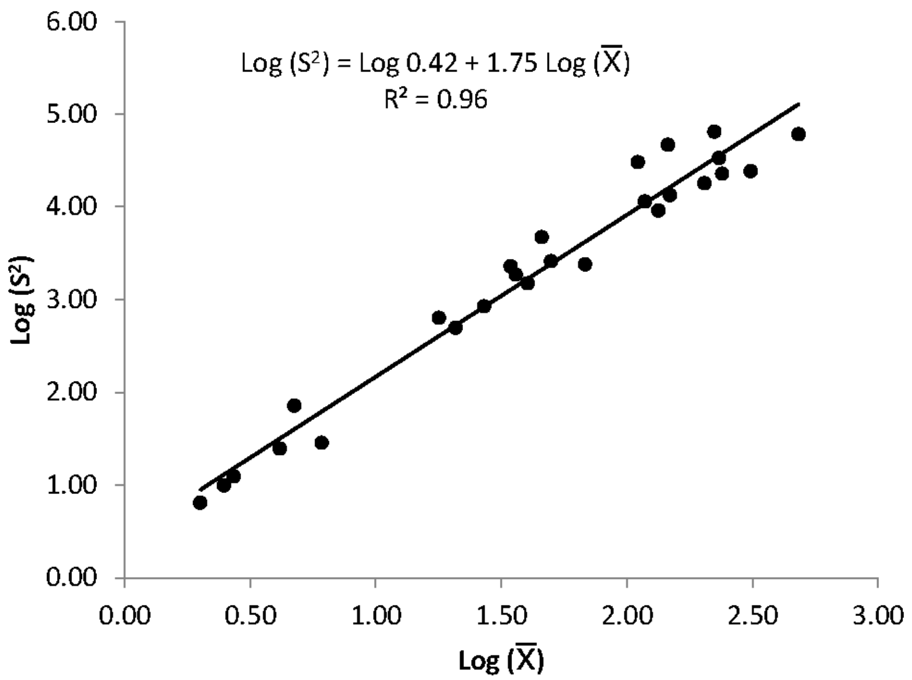

Figure 12. Regression between the variance and the mean of adults of *Trioza erytreae* captured using sticky traps according to the Taylor´s law.

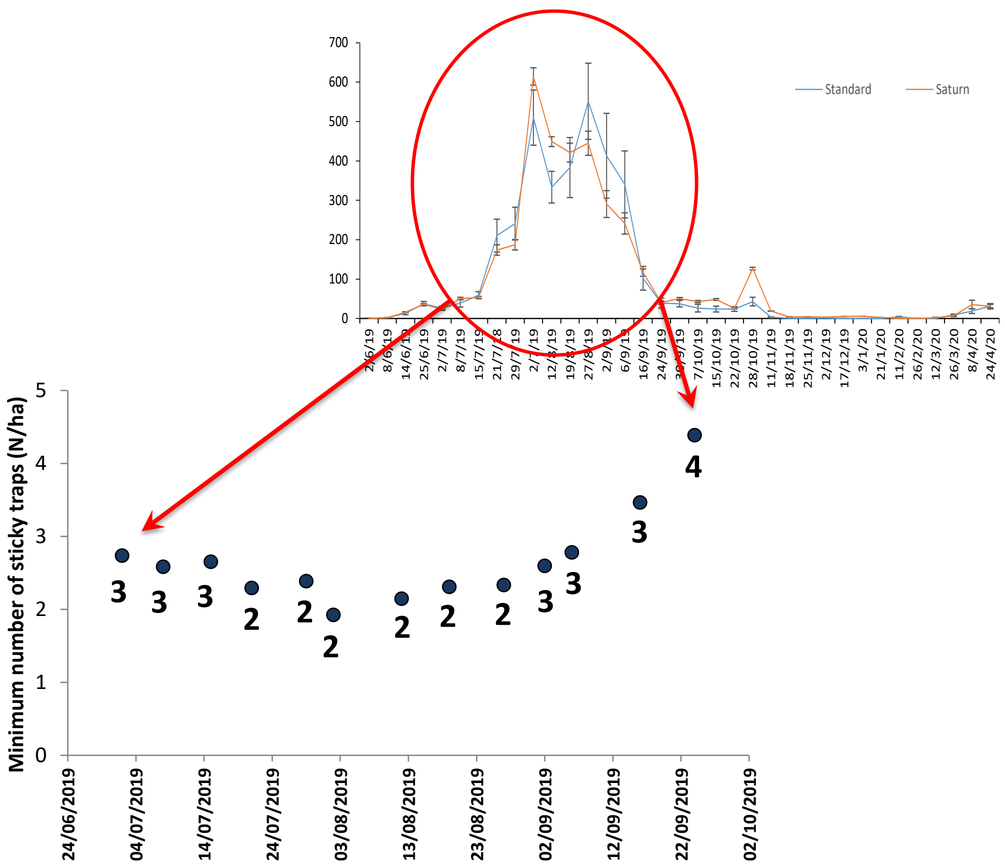

Figure 13. Minimum number of yellow sticky traps per hectare required to obtain 90% of accuracy on the estimation of the population of *Trioza erytreae* during the outbreak.

6 REFERENCES

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