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Efficacy of Selected Insecticides for Chemical Control of the African Citrus Psyllid, *Trioza erytreae* (Psylloidea: Triozidae)

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Abstract: The recent spread of the African citrus psyllid, *Trioza erytreae*, one of the vectors of the devastating citrus disease, Huanglongbing (HLB), to parts of mainland Europe has created considerable concern. In this study, we show the efficacy of several insecticides with varying modes of action on different developmental stages of *T. erytreae*. In laboratory trials, spinetoram caused the highest mortality in *T. erytreae* eggs (between 80 and 90%), while dimethoate, lambda cyhalothrin, spinetoram, cyantraniliprole, and paraffin oil showed over 90% mortality on nymphs. Dimethoate, spinetoram and paraffin oil also demonstrated high efficacy against adults. In winter field conditions, dimethoate showed the best results to control *T. erytreae* nymph populations, and lambda cyhalothrin showed persistent egg control. Our results support the use of different insecticides to control *T. erytreae* for adults in winter, and for egg and nymph populations in spring and summer.

Keywords: chemical control; citrus; insecticides; integrated pest management

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1. Introduction

The African citrus psyllid, *Trioza erytreae* (Del Guercio, 1918) (Hemiptera: Triozidae), is a vector of the phloem-limited bacteria *Candidatus* Liberibacter africanus and *Candidatus* Liberibacter asiaticus. These bacteria are the causal agents of African citrus greening disease [1,2], and Asian citrus greening disease [3], respectively. Both bacteria are etiological agents causing Citrus greening or Huanglongbing (HLB), which is currently the most devastating citrus disease worldwide [4,5]. HLB has been associated with the collapse of several citrus industries in Asia, America [6], and Africa [7,8].

Trioza erytreae is mainly found in Sub-Saharan Africa and its neighbouring islands [9,10]. In 1994, *T. erytreae* was found in the archipelago of Madeira (Portugal) [11] and in 2002 in the Canary Islands [12]. In 2014, *T. erytreae* was detected in north-western Spain (Pontevedra, Galicia) and northern Portugal [13], which was the first time it was detected in the mainland Mediterranean basin. Control measures applied during the outbreak did not stop the spread of the pest, and currently, it is present in several key citrus-producing areas of Portugal, including the region of the Algarve, next to Huelva, a very important

citrus producing area of Spain [10,14,15]. Therefore, a major effort must be made to contain the spread of this pest, which now threatens the nearby citrus production of Andalusia. Indeed, it presents a great risk for the entire Mediterranean citrus sector, since the vector's arrival in an area is usually followed by the arrival of the bacteria [4,6].

Since *T. erytreae* was detected on the Canary Islands none of the generalist predators have been effective in reducing psyllid populations. Recently, a classical biological control program was launched and the parasitoid *Tamarixia dryi* (Waterston) (Hymenoptera: Eulophidae) was imported and released in the area [16].

Therefore, chemical control is a crucial strategy to prevent the spread of this vector and reduce its impact where the disease is already established [17]. However, little information on *T. erytreae* chemical control is available in the literature. Most studies on its control have been carried out in South Africa and were published before the 1990s [18–21]. The main chemical strategy has focused on neonicotinoids, thiamethoxam, and imidacloprid, but nowadays they cannot be used in the Mediterranean citrus area, since they are banned in Europe [22,23]. Moreover, there are serious concerns about resistance development in *T. erytreae* populations. Thus, any chemical control programs against this pest should consider the rotation of active ingredients to be sustainable in the long term [24]. For this reason, there is considerable urgency to develop an effective strategy for the control of *T. erytreae* using different alternatives.

For this reason, we conducted a series of experiments to compare the efficacy of several pesticides under semi-field and field conditions. At the same time, we evaluated the efficacy of some pesticides with different modes of action on different stages of development of *T. erytreae* in the laboratory, either by contact and/or systemic bioassays. Indeed, it is of great importance to evaluate different control strategies for each developmental stage of the pest.

Thus, the objective of the present study is to compare the efficacy of various insecticides through laboratory, semi-field, and field studies to provide useful information for the development of an integrated control strategy against *T. erytreae*.

2. Materials and Methods

2.1. Insecticides

The fifteen insecticides used in the assays are listed in Table 1. For the semi-field and field assays, they were selected as their use was authorized at the time of the assay to control other citrus pests. Imidacloprid and thiamethoxam are broad-spectrum insecticides with high efficacy on pest species of different orders. Both neonicotinoids, although they are currently not authorized in the European Union for outdoor use, they are still used in several other citrus producing countries. For the laboratory assays, insecticides were selected based on their different modes of action, as well as for being effective at controlling other citrus sucking pests or other psyllids in other crops. Selected active ingredients (ai) have either contact action, systemic action, or both. The insecticides were applied at the maximum field recommended concentration (MFRC).

Table 1. Insecticides used in the laboratory bioassays, semi-field and field trials. LC: contact lab bioassay; LS: systemic lab bioassay; SF: semi-field trial; FW: winter field trial; FS: spring field trial.

Trade Name	Active Ingredient	Formulation [a]	IRAC Subgroup [b]	Rate (%)	Manufacter	Type of Trial				
						LC	LS	SF	FW	FS
Gazel Plus®	Acetamiprid	20% SG	4A	0.025	BASF	x	x			
Apache®	Abamectin	1.8% EC	6	0.04	Afrasa			x		x
Sulfocal®	Calcium polysulfide	18.5% SL	UN	0.08	Agrotecnología				x	
Exirel®	Cyantraniliprole	10% SE	28	0.1	FMC	x	x			
Perfekthion Top®	Dimethoate	40% EC	1B	0.1	BASF	x	x	x	x	
Teppeki®	Flonicamid	50% WG	29	0.005	Belchim Crop Protection	x	x			
Sivanto®	Flupyradifurone	20% SL	4D	0.05	Bayer CropScience	x	x			

Confidor®	Imidacloprid	20% SL	4A	0.8	Bayer CropScience			x		x
Kenotrin®	Lambda cyhalothrin	2.5% WG	3A	0.08	Kenogard	x		x	x	x
Ovitex®	Paraffin oil	83% EC	UN	1.5	Belchim Crop Protection	x				
Plenum®	Pymetrozine	50% WG	9B	0.04	Syngenta		x	x		
Delegate®	Spinetoram	25%WG	5	0.04	Corteva Agriscience	x				
Movento 150 O-TEC®	Spirotetramat	15% OD	23	0.04	Bayer CropScience	x	x	x		x
GF-2626®	Sulfoxaflor	12% SC	4C	0.04	Dow Agroscience	x	x			
Actara 25 WG®	Thiamethoxam	25% WG	4A	0.03	Syngenta Crop Protection			x		x

[a] SG: water soluble granules; EC: emulsifiable concentrate; SL: soluble concentrate; SE: suspo-emulsion; WG: water dispersible granules; OD: oil dispersion; SC: suspension concentrate. [b] IRAC (Insecticide Resistance Action Committee): 4A: neonicotinoids; 6: avermectins, milbemycins; UN: compounds of unknown or uncertain IRAC subgroup; 28: diamides; 1B: organophosphates; 29: flonicamid; 4D: butenolides; 3A: pyrethroids, pyrethrins; 9B: pyridine azomethine derivatives; 5: spinosyns; 23: tetronic and tetramic acid derivatives; 4C: sulfoximines.

2.2. Insects

Trioza erytreae eggs, nymphs, and adults used in laboratory bioassays were collected directly from insecticide-free lemon *Citrus limon* (L.) Osbeck orchards on Tenerife (28°29′21.6″ N, 16°21′20.3″ W) and Gran Canaria (28°03′45.9″ N, 15°34′28.9″ W) (Canary Islands, Spain) less than 2 h before performing the experiments. Adults of *T. erytreae* used in the semi-field trial came from a laboratory colony in a greenhouse without climate control on *Citrus sinensis* (L.) Osbeck (Lane Late cultivar; Carrizo citrange rootstock) potted plants.

2.3. Laboratory Bioassays

These bioassays were conducted under laboratory conditions in the Canary Institute of Agricultural Research (ICIA) facilities in Valle de Guerra (Tenerife).

2.3.1. Contact Laboratory Bioassays

Contact assays were conducted with *T. erytreae* eggs, nymphs, and adults. The protocol used was modified from Srinivasan et al. [25]. Citrus shoots heavily infested with *T. erytreae* eggs and nymphs collected from citrus orchards were transported to the laboratory in a portable fridge (approx. 10 °C). Leaves with more than 20 eggs and leaves with 20–60 1st–3rd instar nymphs per leaf were selected. Each replicate consisted of one selected leaf, and four replicates were performed for each treatment and developmental stage. All selected leaves, either with eggs or nymphs, were dipped in the different insecticide solutions (Table 1) for 10 s and placed on a filter paper disc until the complete evaporation of the insecticides. Distilled water was used as a control treatment. After that, each leaf with eggs was placed in a Petri dish (90 mm diameter) containing an agar-agar solution (1.5%) for 96 h, where petioles were inserted to keep leaf turgidity. These Petri dishes had small holes in the lids to allow ventilation and were sealed with Parafilm© (Bemis Company, Inc., Neenah, USA) to prevent the emerging nymphs from escaping. The number of non-hatched eggs and nymphs were counted after this period. Additionally, each leaf with nymphs was placed in a Petri dish (50 mm diameter) with a wet filter paper disc at the bottom, and the numbers of dead and live psyllids nymphs were recorded after 24 h.

Trioza erytreae adults were collected in groups of ten with an insect pooter from citrus groves and taken to the laboratory in a portable fridge. There, each group was chilled for 90 s and then transferred to a Petri dish (50 mm diameter) with a filter paper disc on the bottom. Each insecticide (0.5 mL) was then topically applied to each group of adults with a micropipette. Four replicates per treatment were performed. After two minutes of the application, adult psyllids were transferred to a ventilated plastic cage (Ø = 60 mm, h = 32 mm) containing a single citrus leaf, as a food source, and a small water vial (2 mL) as a humidity source. After 24 h, the numbers of dead and live adults were recorded.

2.3.2. Systemic Laboratory Bioassays

The toxicity of eight systemic insecticides (Table 1) was assessed against nymphs and adults of *T. erytreae* using an uptake bioassay technique for systemic insecticides, as described in [26]. Citrus shoots of 15 cm, with at least two terminal leaves infested with psyllid nymphs, were collected. Each main stem of the shoot was placed in a glass tube (50 mL) with one insecticide solution for 24 h. Distilled water was used as a control treatment. Four replicates were performed per treatment. To prevent nymphs from escaping, each stem was protected with a paper cone with glycerine in the corners. After that, shoots were transferred to a glass tube with water, where nymph mortality was checked after 24 h.

For the adult experiment, citrus shoots were placed in each insecticide solution for 24 h. After that, shoots were transferred to a clean 1.5 mL tube with water. Each tube was placed inside a plastic container (1 L) with a filter paper disc on the bottom, a 2 mL water vial as a humidity source, and ten adults of *T. erytreae*. Four replicates per insecticide were performed. After 48 h, the numbers of dead and live psyllids adults were counted.

2.4. Semi-Field Trial

Potted plants of *C. sinensis* (Lane Late cultivar; Carrizo citrange rootstock) with a height of 100–120 cm were pruned to ensure homogeneity in new flushing and artificially infested with *T. erytreae* adults for three days at the stage of 2–4 cm long shoots. In each plant, three shoots with 1st–2nd instar nymphs of *T. erytreae* were selected, and the remaining unhatched eggs were removed to ensure a minimum of 200 nymphs of similar age per plant. Selected shoots were covered by tulle bags to catch and count emerged adults, and to avoid new egg laying. In all trials, temperature and humidity were recorded using a data logger MC USB-502 (Measurement Computing, Norton, USA) with protective housing throughout the study (Supplementary Materials, Table S1). The trial was set up in May 2017 at an experimental greenhouse of the ICIA.

Thirty-two plants were used in this experiment, which was established as a randomized complete block design, consisting of four replicates of eight treatments (seven insecticides (Table 1) and a control), with one potted citrus plant (experimental unit). The entire surface of the plant was sprayed with 0.2 L of insecticide solution that were applied using a manual sprayer (MATABI 1000 cc, Goizper Group, Antzuola, Spain), thus avoiding excessive dripping. The numbers of live adults and nymphs of *T. erytreae* per shoot were counted one day before and at 3, 7, 14, 21, and 28 days after application (daa). Samples comprised the observation of the three entire marked shoots per plant. Thus, a total of twelve shoots per treatment were observed on each sampling date.

2.5. Field Trial

Three field trials were conducted in different commercial citrus orchards in Tenerife between the coordinates 27°37′ and 29°25′ north latitude and 13°20′ and 18°10′ west longitude.

2.5.1. Winter

The winter trial was set up in February 2017 in a 5000 m² citrus grove of 40-year-old trees (Valencia Late, Washington Navel & Navelina orange cultivars) located in La Orotava and heavily infested by *T. erytreae*. Ninety-six trees were used in a completely randomized block design, consisting of four replicates with six trees per plot. Water was applied to control plots. Three insecticides (Table 1) were assessed for their efficacy in this trial. Foliar sprays were performed with a backpack pump sprayer (MATABI, Super Agro 16, Goizper Group, Antzuola, Spain) equipped with a 1.6 mm spray nozzle. An average of 3.3 L/tree was used.

The numbers of live adults, nymphs, and eggs of *T. erytreae* per shoot were counted one day before application of treatments and at 14, 28, 42, and 62 days afterwards. Samples comprised four entire shoots 5–20 cm long per tree, each one from the four central trees

of the plot. Collected shoots were transported to the laboratory for assessment under a dissecting microscope. The percentage of *T. erytreae* infestation was calculated for the two central trees of each experimental unit by comparing the numbers of infested shoots by *T. erytreae* present inside a plastic ring (0.50 cm diameter) from those susceptible to being infested at 120 cm height and west orientation.

2.5.2. Spring

These experiments were set up in May 2017 and June 2018 in a commercial citrus grove of 11,761 m² with 5-year-old trees (Valencia Late and Washington Navel orange cultivar) in Valle de Guerra. One hundred and sixty-two trees were selected for this experiment, established as a randomized complete block design, consisting of three replicates with each plot (nine trees). Control plots were treated with water. In two central trees per plot, two shoots per tree naturally infested with 1st–2nd instar nymphs of *T. erytreae* were selected. Unhatched eggs were removed to ensure approximately 100–200 individuals of similar age per shoot. All selected shoots were covered by a tulle bag, to catch and count emerging adults and avoid new egg laying.

Five insecticides (Table 1) were assessed for their efficacy in this trial. Foliar sprays were applied with a motor pump sprayer (HONDA GX 100, Honda Motor Co., Hamamatsu, Japan) with 3600 rpm, 25 bar pressure, 2.8 horsepower, equipped with a 1.6 mm spray nozzle. An average of 7 L per tree was applied.

The total numbers of live adults and nymphs of *T. erytreae* per shoot were counted one day before treatment application and at 7, 14, 21, and 28 days afterwards. Samples comprised two entire shoots 5–20 cm long per tree. Collected shoots per treatment on each sampling date were transported to the laboratory for assessment under a Nikon SMZ1270 dissecting microscope (Nikon, Tokyo, Japan).

The percentage of nymph mortality (semi-field and spring trials) was calculated indirectly through survival (100% survival), which we calculated as a ratio between the number of live nymphs plus the number of emerged adults at the end of the trial and the initial number of nymphs.

2.6. Statistical Analysis

In the laboratory bioassays, percentage mortality was calculated in all contact and systemic bioassays for each *T. erytreae* stage treated. This percentage was compared with the control using the t-test ($p < 0.05$) proc TTEST (SAS, 2009, SAS Institute Inc, Cary, NC, USA) in the laboratory contact bioassays. In the laboratory, systemic bioassays percentage mortality was analysed using a one-way analysis of variance with the treatment as a factor and mortality as the dependent variable proc ANOVA (SAS, 2009, SAS Institute Inc, Cary, NC, USA) followed by a Tukey post-hoc test.

Mortality percentages of the contact bioassay and systemic with nymphs were corrected by the Henderson-Tilton formula [27], since the initial number of individuals was not uniform among treatments. On the contrary, since the population of adults in the systemic bioassays was uniform among treatments, we applied Schneider-Orelli's formula [28].

For the semi-field and field trials, the percentages of nymph mortality were transformed to arcsine (sqrt (% of mortality)) before performing an analysis to stabilize the variance. Data were analysed using one-way analysis of variance (ANOVA) followed by separation of means with Tukey's least significant difference (LSD) test. Data that did not meet normality requirements after transformations were analysed using the Kruskal-Wallis non-parametric test and Dunn's non-parametric multiple comparison test. All statistical tests were conducted at the 0.05 level of significance using Statistix 10 software (Analytical Software, Tallahassee, FL, USA). When significant differences were found, mortality was corrected using Abbott's equation [29], after verifying before the experiments that the pest populations in the trees assigned to the different treatments were not significantly different.

3. Results

3.1. Laboratory Bioassays

3.1.1. Contact Bioassays

In the contact bioassays, only four active ingredients were effective on eggs: dimethoate, lambda cyhalothrin, spinetoram, and flupyradifurone (Table 2). The efficacy on eggs ranged from 64.2% for flupyradifurone to 81% for spinetoram. In the case of nymphs, three insecticides were ineffective (flupyradifurone, spirotetramat, and pymetrozine), whereas efficacy ranged from 60% for sulfoxaflor to 100% for paraffin oil. Four active ingredients (paraffin oil, dimethoate, lambda cyhalothrin, and spinetoram) showed efficacies > 93%. Six active ingredients were effective on adults, ranging from 54.2% for flupyradifurone to 100% for dimethoate, paraffin oil, spinetoram, and dimethoate presented efficacies > 95%.

Table 2. Percentage of corrected mortality (%) in *Trioza erytreae* eggs, nymphs and adults in contact bioassays. The mortality was recorded 96 h after treatment for eggs, and 24 h after treatment for nymphs and adults.

Active Ingredient	Eggs	t	Nymphs	t	Adults	t
Acetamiprid	0	2.44	69.4	6.12 *	78.4	8.99 *
Cyantraniliprole	0	1.44	65.1	8.97 *	0	0.38
Dimethoate	71.6	3.23 *	93.7	15.41 *	100	19.66 *
Flonicamid	0	0.90	62.9	6.20 *	0	0.10
Flupyradifurone	64.2	2.69 *	0	2.02	54.2	4.39 *
Lambda cyhalothrin	65.8	3.06 *	99.2	7.86 *	79	6.47 *
Paraffin oil	0	0.80	100	20.45 *	95.8	2.85 *
Pymetrozine	0	0.43	0	0.86	0	0.60
Spinetoram	81	4.29 *	95.8	18.18 *	95.8	2.85 *
Spirotetramat	0	1.50	0	0.41	0	0.75
Sulfoxaflor	0	0.50	60	10.39 *	0	0.90

df = 6. * Indicate significant differences ($p < 0.05$; t-test) between treatments and the control.

The active ingredients dimethoate, lambda-cyhalothrin, and spinetoram were significantly effective on the three development stages of *T. erytreae*. Dimethoate was 100% effective in adults, over 90% in nymphs, and 71.6% in eggs. Lambda cyhalothrin showed high efficacy against nymphs, almost 100%, 78.9% in adults, and 65.7% in eggs. Regarding spinetoram, the percentage of efficacy was the same for nymphs and adults, 95.8%, and 81% in eggs.

Paraffin oil was highly effective against nymphs with 100% mortality and 95.7% mortality in adults, but it was not significantly effective against eggs. The same scenario was repeated for acetamiprid, though mortality was lower, 69.4% in nymphs and 78.3% in adults. Flupyradifurone was effective in eggs and nymphs, but with lower mortality in comparison with the other active ingredients, 64–21% in eggs and 54.1% in nymphs. Cyantraniliprole, sulfoxaflor, and flonicamid were only significantly effective for nymphs; with around 60% mortality. Finally, there were two active ingredients, spirotetramat and pymetrozine, which were not effective against any of the three stages of *T. erytreae* in contact bioassays.

3.1.2. Systemic Bioassays

In the systemic bioassays (Table 3), five of the eight active ingredients tested were significantly effective against nymphs, however, only dimethoate was effective against adults (90.6%). For nymphs, dimethoate and cyantraniliprole led to mortality of more than 90%, while with sulfoxaflor and spirotetramat mortality was higher than 75% and flupyradifurone led to 51.7% mortality. Dimethoate was effective on all the developmental stages,

while cyantraniliprole and sulfoxaflor showed efficacy only against nymphs, both applied by contact and systemically.

Table 3. Mean percentage of *T. erytreae* nymph mortality (%) ± standard error in systemic bioassays. In brackets the mean percentages of corrected mortality (%). The mortality was recorded 48 h after shoots were treated.

Active Ingredient	Nymphs	Adults
Control	19.4 ± 7.5 a	7.5 ± 4.8 A
Acetamiprid	49 ± 3.9 ab (0)	27.5 ± 8.5 A (0)
Cyantraniliprole	95.4 ± 1.9 c (93.7)	5 ± 5 A (0)
Dimethoate	96 ± 3.4 c (94.5)	80 ± 4.1 B (90.6)
Flonicamid	46.6 ± 6.7 ab (0)	12.5 ± 6.3 A (0)
Flupyradifurone	57.9 ± 5.1 b (51.8)	15 ± 8.7 A (0)
Pymetrozine	50.9 ± 8.2 ab (0)	5 ± 5 A (0)
Spirotetramat	73.9 ± 13.3 bc (77.1)	5 ± 2.9 A (0)
Sulfoxaflor	79.3 ± 7.8 bc (78.8)	22.5 ± 8.5 A (0)
F	12.35	14.36
df	8, 27	8, 27
p-value	<0.001	<0.001

Means followed by different letters in the same column differ at the 5% significance level when compared using the Tukey test (lowercase for nymphs, capital letters for adults).

3.2. Semi-Field Trial

No phytotoxic symptoms were observed in any of the trials. In this trial, all active ingredients assayed significantly reduced *T. erytreae* nymph densities (Table 4). Dimethoate was the most effective in both the short (3 daa) and long term (28 daa), showing percentages of nymph mortality ranging from 85% to almost 100%, respectively. Thiamethoxam, imidacloprid, and lambda cyhalothrin showed similar mortalities to dimethoate at the end of the trial, but with lower knockdown effects. Lambda cyhalothrin showed significantly less nymph mortality than dimethoate at 3 and 7 daa (65.6 and 74.2%, respectively) but reached 93.6% of nymph mortality at 28 daa. Spirotetramat and pymetrozine initially showed a similar effect on *T. erytreae* nymphs, with mortalities of 48.1% and 37.6%, respectively. Nevertheless, in the case of spirotetramat, the mortality observed increased up to 90.6% at 28 daa, while pymetrozine reached only 70.5% mortality, and was not significantly different from that observed in the untreated control. Finally, abamectin showed the lowest efficacy in reducing *T. erytreae* nymphs at 3, 7, 14, and 21 daa (less than 50%), although it was not significantly different from imidacloprid, thiamethoxam, spirotetramat, and lambda cyhalothrin at 28 daa (91.2%). Thus, six of the seven active ingredients tested were significantly effective against *T. erytreae* nymphs by the end of the trial; with only the active ingredient pymetrozine producing less than 90% efficacy.

Table 4. Mean percentage of *T. erytreae* nymph mortality (%) ± standard error at different times (3, 7, 14, 21, and 28 days after application (daa)) in the semi-field trial. In brackets the mean percentages of corrected mortality (%).

Active Ingredient	3 Daa	7 Daa	14 Daa	21 Daa	28 Daa
Control	2.4 ± 1.8 a	8.3 ± 4.5 a	22.3 ± 10.9 a	33.0 ± 11.6 a	47.8 ± 19.0 a
Abamectin	16.4 ± 7.7 b (14.3)	25.4 ± 9.7 ab (0)	35.2 ± 9.5 ab (0)	42.8 ± 8.5 ab (0)	91.2 ± 5.5 bc (79.5)
Dimethoate	85.2 ± 1.5 e (84.8)	88.9 ± 2.3 e (84.8)	89.7 ± 2.1 e (86.5)	90.5 ± 1.4 d (85.5)	99.5 ± 0.9 c (98.5)
Lambda cyhalothrin	65.6 ± 4.9 d (64.9)	74.2 ± 7.6 d (71.3)	77.5 ± 9.6 de (70.9)	78.6 ± 8.6 d (66.9)	93.6 ± 4.6 c (84.6)
Pymetrozine	37.7 ± 3.8 c (38.8)	51.5 ± 5.5 c (59.3)	53.3 ± 8.4 bc (40.9)	54.1± 8.8 b (32.5)	70.5 ± 7.8 ab (0)
Spirotetramat	48.1 ± 3.1 c (49.9)	53.4 ± 4.3 c (52.6)	61.8 ± 1.6 cd (50.0)	62.4 ± 1.4 bc (43.0)	90.6 ± 6.4 bc (79.5)
Imidacloprid	72.2 ± 4.9 de (71.4)	74.7 ± 3.5 d (71.5)	76.3 ± 2.1 de (68.9)	80.4 ± 3.5 d (70.7)	99.1 ± 0.7 c (98.2)
Thiamethoxam	73.6 ± 7.5 de (72.9)	77.9 ± 5.5 de (75.1)	82.1 ± 2.1 e (76.8)	85.2 ± 3.4 d (77.6)	99.3 ± 0.4 c (98.5)

F	93.80	67.62	30.93	24.01	18.86
df	(7, 24)	(7, 24)	(7, 24)	(7, 24)	(7, 24)
p-value	⩽0.001	⩽0.001	⩽0.001	⩽0.001	⩽0.001

Means followed by different letters in the same column differ at the 5% significance level when compared using the Tukey test.

3.3. Field Trials

3.3.1. Winter

Significant differences between treatments and untreated control were only observed at the first assessment (14 daa) (Table 5). Dimethoate and calcium polysulphide showed the highest nymph mortality (0.5 and 0.1 live nymphs/shoot, respectively) followed by Lambda cyhalothrin (3.2 live nymphs/shoot). After 14 days of treatment, all active ingredients tested showed similar mortalities of *T. erytreae* nymphs, and no statistical differences were found between them or with the control. Nevertheless, Lambda cyhalothrin showed the greatest persistence in reducing *T. erytreae* nymph population. Regarding *T. erytreae* egg control (Table 5), a similar trend was observed, since, at the first assessment, all active ingredients were effective, but after that, the lambda cyhalothrin treatment was the only one that significantly reduced the number of live eggs per shoot compared to the untreated control throughout the trial. Dimethoate and lambda cyhalothrin decreased the percentage of infested shoots at 14 and 28 daa, but only dimethoate significantly reduced this infestation at the end of the trial in spring (Table 5).

Table 5. Number of *T. erytreae* eggs and nymphs per shoot (mean± standard error) and percentages of infested shoots at different times (14, 28, 48, and 62 days after application (daa)) in 2017 winter trial. In brackets, the percentage means of corrected mortality.

Active Ingredient		**Individuals per Shoot**				**Infested Shoots**			
		14 Daa	**28 Daa**	**48 Daa**	**62 Daa**	**14 Daa**	**28 Daa**	**48 Daa**	**62 Daa**
Control	Eggs	122.2 ± 40.4 a	128.2 ± 25.3 a	369.3 ± 76.3 a	524.8 ± 103.9 a	57 ± 5.2 *a*	86.7 ± 8.6 *a*	100 ± 0 *a*	100 ± 0 *a*
	Nymphs	32.7 ± 12.0 A	17.7 ± 5.8 A	81.8 ± 5.3 A	103.1 ± 18.8 A				
Dimethoate	Eggs	16.9 ± 5.9 b (89.5)	110.4 ± 16.2 a (0)	284.7 ± 69.4 ab (0)	408.4 ± 102.7 ab (0)	23.3 ± 4.6 *b*	57.5 ± 10.5 *b*	78.8 ± 10.6 *ab*	73 ± 12.2 *b*
	Nymphs	0.5 ± 0.2 C (99.7)	14.1 ± 3.9 A (0)	53.4 ± 18.9 A (0)	62.1 ± 31.6 A (0)				
Calcium polysulphide	Eggs	17.4 ± 2.7 b (92.8)	25.94 ± 15.4 b (19.2)	281.1 ± 79.3 ab (0)	425.2 ± 106.9 ab (0)	60.5 ± 8.1 *a*	81.9 ± 5.8 *a*	97.9 ± 2.1 *a*	97.8 ± 2.2 *ab*
	Nymphs	0.1 ± 0.06 C (98.7)	2.6 ± 0.9 A (0)	39.9 ± 16.6 A (0)	62.1 ± 31.6 A (0)				
Lambda cyhalothrin	Eggs	10.7 ± 3.6 b (89.4)	34.0 ± 13.2 b (72.3)	92.3 ± 17.5 b (70.3)	119.6 ± 21.6 b (72.3)	11.4 ± 4.8 *b*	27.9 ± 8.4 *c*	73.6 ± 11.7 *b*	85.5 ± 2.1 *ab*
	Nymphs	3.2 ± 1.2 B (91.9)	14.4 ± 10.7 A (0)	26.9 ± 7.4 A (0)	28.7 ± 9.9 A (0)				
F	Eggs	4.43	12.90	5.24	4.82	17.61	19.62	4.01	4.76
	Nymphs	49.86	1.49	2.41	2.08				
df	Eggs	(3, 9)	(3, 9)	(3, 9)	(3, 9)	(3, 60)	(3, 60)	(3, 51)	(3, 60)
	Nymphs	(3, 9)	(3, 9)	(3, 9)	(3, 9)				
p-value	Eggs	0.03	0.001	0.02	0.03	<0.001	<0.001	<0.05	<0.05
	Nymphs	< 0.001	n.s.	n.s.	n.s.				

Means followed by different letters in the same column differ at the 5% significance level when compared using the Tukey test (lowercase for eggs, capital letters for nymphs and italics for infested shoots). n.s.: non-significant.

3.3.2. Spring

In 2017, thiamethoxam was the most effective pesticide to control *T. erytreae* nymphs in the spring field trial (Table 6), followed by imidacloprid and lambda cyhalothrin. Imidacloprid showed knockdown effects, with less than 10 live nymphs/shoot at 7 daa, while Lambda cyhalothrin led to significantly fewer live nymphs than spirotetramat and imidacloprid at 28 daa, with no significant differences being observed between these last

two. Abamectin was the least effective active ingredient and treated trees did not show significant differences in the number of live nymphs per shoot compared to the untreated control during the trial.

Table 6. Effect of insecticides on *T. erytreae* expressed as the mean of live nymphs per shoot (mean ± standard error) at different times (7, 14, 21, and 28 days after application (daa)) in 2017 and 2018 spring trial. In brackets, the percentage means of corrected mortality (%).

Active Ingredient	2017				2018			
	7 Daa	14 Daa	21 Daa	28 Daa	7 Daa	14 Daa	21 Daa	28 Daa
Control	86.6 ± 1.2 a	91.9 ± 10.6 a	105.4 ± 27.1 a	80.4 ± 37.9 a	25.2 ± 10.1 a	45.0 ± 7.7 a	54.0 ± 17.4 a	56.2 ± 8.8 a
Abamectin	79.5 ± 14.7 a (0)	56.8 ± 7.0 a (0)	44.9 ± 12.2 ab (0)	43.1 ± 22.9 ab (0)	69.1 ± 3.6 bc (49.1)	65.6 ± 15.6 ab (0)	71.7 ± 15.6 ab (0)	79.18 ± 7.2 ab (0)
Imidacloprid	9.1 ± 0.5 c (89.5)	19.4 ± 8.5 b (75.8)	15.2 ± 5.8 bc (86.1)	21.7 ± 16.9 ab (0)	88.9 ± 7.9 c (83.2)	92.2 ± 6.9 c (92.9)	94.9 ± 5.6 bc (93.9)	95.8 ± 5.5 bc (94.9)
Lambda cyhalothrin	20.1 ± 0.3 b (76.8)	18.6 ± 1.9 b (79.3)	19.7 ± 1.8 bc (78.2)	1.9 ± 4.8 b (89.0)	77.7 ± 16.4 bc (83.4)	79.5 ± 13.6 bc (74.8)	85.1 ± 20.9 abc (0)	92.6 ± 7.1 bc (89.6)
Spirotetramat	59.1 ± 11.6 a (0)	41.4 ± 5.8 ab (0)	30.0 ± 7.0 bc (64.8)	44.8 ± 22.3 ab (0)	55.7 ± 18.9 ab (0)	84.2 ± 9.3 bc (32.7)	91.1 ± 3.9 abc (0)	84.9 ± 9.1 b (58.3)
Thiamethoxam	10.4 ± 1.5 c (88.0)	5.2 ± 0.6 c (94.1)	8.4 ± 1.0 c (91.3)	1.8 ± 0.9 b (96.4)	94.6 ± 2.9 c (86.2)	98.5 ± 0.9 c (96.8)	99.5 ± 0.4 c (97.7)	99.7 ± 0.1 c (98.9)
F	61.76	21.84	11.48	4.35	13.20	12.11	6.44	13.84
df	(5, 12)	(5, 12)	(5, 12)	(5, 12)	(5, 12)	(5, 12)	(5, 12)	(5, 12)
p-value	< 0.001	< 0.001	< 0.001	0.017	< 0.001	< 0.001	< 0.005	<0.001

Means followed by different letters in the same column differ at the 5% significance level when compared using the Tukey test.

In 2018, a similar trend to the previous year was observed (Table 6). Thiamethoxam and imidacloprid showed similar effectiveness on *T. erytreae* nymphs and were persistent over time (98.9% thiamethoxam and 94.9% imidacloprid, at 28 daa). Nymph mortalities above 75% in the lambda cyhalothrin treatment were always obtained, ranging from 74.8% at 14 daa to 89.6% at 28 daa. Spirotetramat and abamectin were the least effective on *T. erytreae* nymph control.

4. Discussion

In this study, we have investigated the efficacy of fifteen products that could be candidate insecticides in Integrated Pest Management programs to control *T. erytreae* populations. Such insecticidal vector control is considered one of the main strategies to slow the spread of HLB once the disease is established [17].

Key factors to consider when attempting to control *T. erytreae* populations are the flushing rhythm and flushing quality of the citrus plants, as reported in South Africa [30]. For this reason, the management of this pest must focus on the main flushes in spring, summer, and fall. Although insecticide application in winter effectively decreased nymph density for a short period, it failed to maintain populations at low levels in the long term, such as in the following spring flushes. Therefore, insecticide applications in winter must be applied just to adults, when no new flushes are present that could allow *T. erytreae* adults to overwinter hidden/protected in the tree canopy and feed on mature leaves [31]. In the laboratory adult experiments, six insecticides exceeded 50% efficacy, and three of them (dimethoate, spinetoram, and paraffin oil) even reached 90%. Dimethoate, lambda cyhalothrin, and calcium polysulphide provided good control in winter field assays. The application of these insecticides in this season can also control other citrus pests. For example, paraffin oil applied in winter can control red spider mites, citrus leafminers, and wax scales [32,33]. In addition, application in winter also reduces the potential negative impact of the pesticide treatments on the beneficial entomofauna [34].

Spring flushes tend to be the most abundant and concentrated, and psyllid populations grow very fast during these flushes. Sometimes, a second flushing period occurs in summer and autumn. At these times, treatments should focus on eggs, and especially on

nymphs. In the laboratory assays, four active ingredients showed more than 50% efficacy against *T. erytreae* eggs, but only one, spinetoram, achieved 80–90%. Several of the tested pesticides were very effective in the control of nymphs. All the insecticides tested in the laboratory, except pymetrozine, were effective, and five of them (dimethoate, lambda cyhalothrin, spinetoram cyantraniliprole, and paraffin oil) produced more than 90% nymph mortality. Dimethoate and lambda cyhalothrin also showed high efficacies in the semi-field trial. For *D. citri* nymphs and adults, spinetoram, spirotetramat, cyantraniliprole, sulfoxaflor, lambda cyhalothrin and paraffin oil (only for nymphs) have also been reported as highly effective [35–38]. In addition, cyantraniliprole showed antifeeding activity on *D. citri* adults, which leads to a reduction in HLB transmission [39].

In citrus growing areas where *T. erytreae* and *D. citri* are present, neonicotinoids have been widely used [38]. In the present study, imidacloprid and thiamethoxam were effective against *T. erytreae* nymphs, and persistent over semi-field and field trials. However, both insecticides were recently banned in Europe, as they are considered harmful for pollinators, whose abundance has been greatly reduced when these insecticides were included in chemical pest management programs [40]. As an alternative, neonicotinoid acetamiprid has shown low toxicity in laboratory bioassays. Interestingly, it was effective on nymphs when applied topically but not when applied systemically.

Even though sulfoxaflor, flonicamid, flupyradifurone, and spirotetramat showed medium efficacy (50–80%) on *T. erytreae*, they can also be used in Integrated Pest Management programs, since they act on other citrus pests such as aphids, citrus leafminer, or whiteflies [41–43]. Moreover, citrus entomofauna is rich and diverse, thus, giving these natural enemies an important role to play in conservation biological control of citrus pests [44]. Owing to this, the toxicity of selected insecticides for *T. erytreae* populations on predators and parasitoids present on citrus must also be considered [45]. Dimethoate, which showed the highest efficacy against *T. erytreae* in our work, reported the most negative effect on *T. erytreae* parasitoid, *T. dryi* (Dionisio, 2021). However, some of the tested insecticides, such as cyantraniliprole were reported to be much less toxic to *T. dryi* than to the target psyllid [46]. Similarly, this insecticide showed less toxicity to the *D. citri* parasitoid, *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) than to the target psyllid [37]. Additionally, sulfoxaflor was reported as less toxic for this parasitoid than for *D. citri* adults [47]. Moreover, in Florida, lacewing abundance was not affected by insecticide treatments against *D. citri*, even when broad-spectrum insecticides were applied [48]. In the same study, however, the reduction of spider predation on *D. citri* in spring could be explained by the broad-spectrum insecticides used in winter, though insecticides used later such as spinetoram and spirotetramat were not considered toxic to spiders.

Resistance of *D. citri* to different chemical families has been reported for chlorpyrifos (organophosphates), fenpropathrin (pyrethroids), imidacloprid, and thiamethoxam (neonicotinoids). Therefore, it is crucial to contemplate the need to rotate between different modes of action to maintain effective control of psyllid pests [24].

In conclusion, the first tool to slow the spread of *T. erytreae* in a new citrus growing area is the application of chemical treatments. In this work, we have shown that several phytosanitary products with different modes of action have high efficacy and can be used on citrus trees for the control of *T. erytreae*. Specifically, for the best results from chemical control of *T. erytreae* consideration must be given to the plant phenology, the season of the year, and the main developmental stages of the pest. Such chemical control integrated into pest management programs of citrus fruits could also be effective for the control of other pests, yet preserving, as far as possible, the natural enemies that are present naturally or released in citrus groves.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/article/10.3390/agronomy12020441/s1, Table S1: Weather conditions registered through experiments in semi-field and field trials.

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