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Life Vida for citrus

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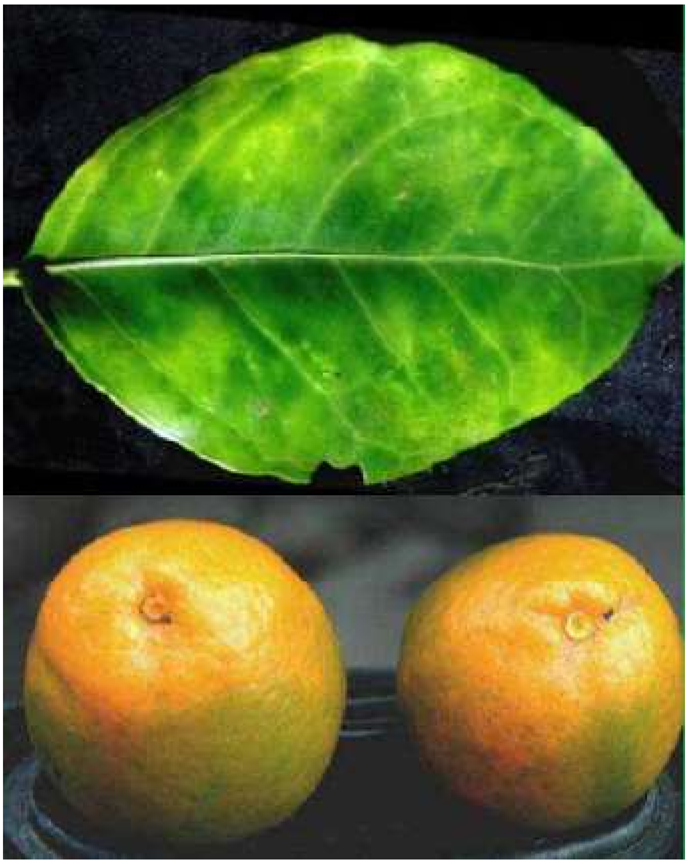
1. Introduction to Huanglongbing (HLB)

Introduction to Huanglongbing (HLB) or citrus greening



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1.1- Introduction

Citrus greening, also known as Huanglongbing (HLB) or yellow dragon disease, poses a major threat to citrus farming worldwide. This disease, described as the most devastating of citrus fruits, reduces the yield and quality of the fruit, causing the death of the tree in a period of 3-5 years.

HLB disease is caused by bacteria (Candidatus Liberibacter spp.) That live and multiply in the tree's phloem. These bacteria are transmitted to other healthy trees by two psyllids, Diaphorina citri and Trioza erytreae, as well as by grafting with infected plant material.

Until relatively recently, only two citrus zones in the world have been free of HLB and its vectors (Diaphorina citri and Trioza erytreae): Australia and the Mediterranean Basin (Bové and Durán-Vila, 2016). However, the situation in Europe is currently seriously threatened after the recent detection of Trioza erytreae in the Iberian Peninsula (Spain and Portugal; Pérez-Otero et al., 2015)

There is currently no cure for HLB disease, the best control strategy being the prevention of the disease, establishing measures that shield the sector from the entry of plants and plant materials from areas affected by both HLB disease or its vector. In this sense, the information and awareness of the different agents of the sector, as well as of the general public, is of vital importance in the early detection of the pest-vector and / or of the disease and its effective eradication in the case of that reached the citrus regions of the Mediterranean. Thus, disease prevention requires an integrated approach: the unique use of certified clean plant material; regular monitoring of trees for the detection and control of the disease vector or symptoms; as well as the notification to the competent authority of each country or region of the suspicion of HLB or presence of any of its vectors.

On the other hand, HLB control and management techniques carried out in countries where the disease is present, consisting of the massive application of insecticidal treatments (20 or more per year), are incompatible with the production systems, the demands of the European distribution and regulation, the latter very restrictive in the use of active and demanding materials in the maximum residue limits. Thus, in Europe, there is currently no authorized product for psila control, so it is necessary to develop sustainable control strategies, based on the rational management of insecticides, as well as biological and technological control: cultivation techniques and plant material tolerant / resistant to HLB.

In this sense, according to different authors, an effective and sustainable long-term HLB management program requires the use of tolerant or resistant plant material (Albrecht and Bown, 2012; Grosser and Gmiter, 2014). As with the variety, a different level of susceptibility to HLB has been found among employers (Folimonova et al., 2009; Albrecht and Bown, 2011; Grosser and Gmiter, 2014).

1.2.- African citrus psila, *Trioza erytreae*

1.2.1.- Taxonomy

The African citrus psila, Trioza erytreae, is a hemiptera insect of the Triozidae family.

1.2.2.- Geographical distribution and ecological requirements

Originally from sub-Saharan Africa, it has spread to the Arabian Peninsula and the archipelagos that surround the African continent: Reunion, Mauritius, Madeira and the Canary Islands. Its presence in the Iberian Peninsula was confirmed in August 2014 (Pérez-Otero et al., 2015).

The African citrus psila finds the optimal conditions for its development in humid and temperate climates. The combination of high temperatures and low humidity is very detrimental to the development of their populations, causing high levels of mortality in eggs and immature stages.

1.2.3.- Hosts

Like other psilas, it has a relatively restricted host range, including only species of the Rutaceae family. Among these, in addition to the members of the Citrus genus, Clausenaanisata, Vepris undulata and Zanthoxylum (= Fagara) capense are cited.

Within citrus fruits, the Citrus lemon species is one of their favorites. In fact, the first detections of this psila usually take place on lemon.

1.2.4.- Description of the pest

- Adults (Image 1). Body length of approximately 4 mm, light green initial color that at maturity turns a dark hue. Transparent wings, without spots, with a very marked venation. Head and black antennae, except the first segment that is whitish in appearance. When they feed they adopt a very characteristic position, raising the body an angle of 35 ° with respect to the food substrate.

- Nymphs (Image 2). Nymphs of yellowish color, flattened or oval shape and surrounded by a band of waxy filaments. When feeding on the leaves, they inject toxic substances that induce the formation of concave bulges (nests) on the underside of the leaf, inside which they are placed individually. These nests on the underside correspond to bulges or bumps in the bundle of the leaves.

  When populations are high, the leaf and the bud can have a totally deformed appearance.

- Eggs (Image 3). The eggs are yellowish and elongated, which are fixed vertically to the leaf through a small peduncle present at one of its ends.

Image 1.- **Adult**

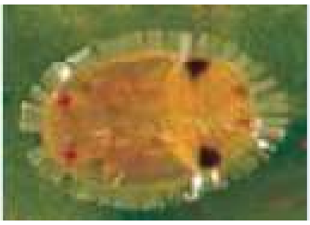


Image 2.- **Nymph**

Imagen 3.- **Eggs**

1.2.5.- Damage to citrus cultivation

The adults of Trioza erytreae, although they can feed on mature leaves, tend to place themselves on developing shoots to feed on a sap of high nitrogen content and thus satisfy their reproductive needs. Thus, their reproduction cycles are closely linked to the budding periods of their host plants.

As already mentioned, the nymphs cause the appearance of gills in the form of depressions or `nests' on the underside of the leaf (A) that corresponds to the presence of very characteristic protrusions in the beam (B), sign very visible and unambiguous of the presence of this vector (Image 4), although of little economic impact.

Image 4.- **Leaf Symptomatology. A: Nests on the underside and B: Beam bumps**

The main damage caused by the African psila is related to the transmission of Ca. Liberibacter africanus, the bacterium associated with the African HLB, a disease that reduces the yield and quality of the fruit, causing the death of the tree in a period of 3- 5 years.

3.- Citrus greening or HLB

1.3.1.- Citrus greening disease or HLB

It is a pathology of great economic impact in most of the citrus areas in which it is established (Image 5). It is estimated that around 100 million trees worldwide are affected by Huanglongbing (HLB).

In Florida, it has caused the loss of more than 4.5 billion dollars and more than 8,000 jobs related to the citrus sector between 2005 and 2011.

Image 5.- **Citrus plantation affected by HLB**

The organisms associated with the disease are gram-negative bacteria (α-Proteobacteria) that live in intimate association with the phloem cells of their host plants, capable of surviving and reproducing inside a vector of the Psylloidea superfamily.

Within these bacteria, Candidatus Liberibacter spp., Several forms or species have been described with differences in their geographical distribution, ecological adaptation and pathogenic capacity.

Specifically, 3 species associated with citrus have been described:

a) Ca. Liberibacter africanus (Laf): present in Africa and the Arabian Peninsula. Considered sensitive to high temperatures (temperature range suitable for the expression of symptoms of African HLB: 25-30 ° C). In regions of warm weather, its incidence is restricted to regions of a certain altitude.

b) Ca. Liberibacter asiaticus (Las): widely distributed throughout the Asian continent and since the last decade present in the main citrus areas of the American continent: Brazil (2004), Florida (2005), Central America and the Caribbean (2008), Mexico (2009), Argentina (2012), Texas (2012) and California (2012).

Considered as tolerant of high temperatures (temperature range suitable for the expression of Asian HLB symptoms: 25-40 ° C).
The pathology associated with the Asian HLB is currently the most serious and economically important in the cultivation of citrus fruits worldwide.

c) Ca. Liberibacter americanus (Lam): First cited in Brazil in 2004 and in the state of Texas (USA) in 2013. Like Laf, it is considered sensitive to high temperatures.

The only citrus zones still free of the disease are Australia, New Zealand, the Middle East and the Mediterranean Basin.

1.3.2.- Symptoms

HLB causes serious growth alterations and significant quality and production losses in the affected trees. If control measures are not taken, infected trees can become dead within 3-5 years.

In general, young trees are the most affected showing a faster development of symptoms.

In the initial stages of the disease, symptomatic trees are characterized by presenting one or several yellow buds that show on the green tree canopy. Over time, these buds evolved giving rise to yellow or pale green branches. In more advanced stages of the disease, various yellow branches began to occupy the entire tree canopy and express other symptoms also associated with the disease:

• Defoliation: poor foliage, leading to “open” growth

• Regressive Death of Branches

• Symptoms in leaves and fruits

One of the most characteristic symptoms of HLB is known as "blotchy mottle" or asymmetric mottling of the leaves (Image 6). Unlike other foliar chlorosis, in the characteristic asymmetric mottling of HLB the different shades (yellow, light green, dark green) of the affected leaves are mixed together, with no clear boundaries between them. In addition, they appear asymmetrically distributed on both sides of the central nerve of the leaf.

Image 6.- **Symptoms of HLB in citrus leaves**

It is during autumn and winter when the expression of this foliar symptom is more pronounced. In the initial phase of HLB disease, asymmetric mottling is usually the only observable foliar symptom, and there are symptoms of zinc deficiencies already in the most advanced stages of the disease.

During the spring and summer months, with a more limited expression of mottled symptoms, the existence of these deficiency symptoms may be the only indication of the presence of the disease in infected trees.

Unlike the lack of nutritional-type zinc, which generally shows a pattern of regular distribution within the plantation, the lack of zinc caused by the HLB usually appears sectorally distributed within the plantation, being found only in some trees.
Another characteristic feature at the foliar level of the disease is that the leaves with asymmetric mottling usually have an erect growth, forming a closed angle with respect to the bud.

Symptoms in adult trees do not appear until 2 to 4 years after infection, making it difficult to diagnose early and monitor and control the disease.

The fruits of citrus trees affected by the HLB present a series of anomalies that greatly reduce their economic and commercial value. These symptoms in fruits are:

- Deformed and small fruits (Image 7)

- Bitter taste

- Color inversion in the external ripening of the fruit, the change in color begins with the peduncular area of the fruit, keeping the green color the area of the bark closest to the stylistic zone.

- Abnormal coloration in the vascular bundles of the peduncular end. The removal of the peduncle in the green fruits of the affected trees reveals a bright orange color on the fruit, which in the case of healthy fruits would be pale green.

- Aborted black-brown colored seeds (Image 7)

- Strong falls of fruits on trees affected by HLB

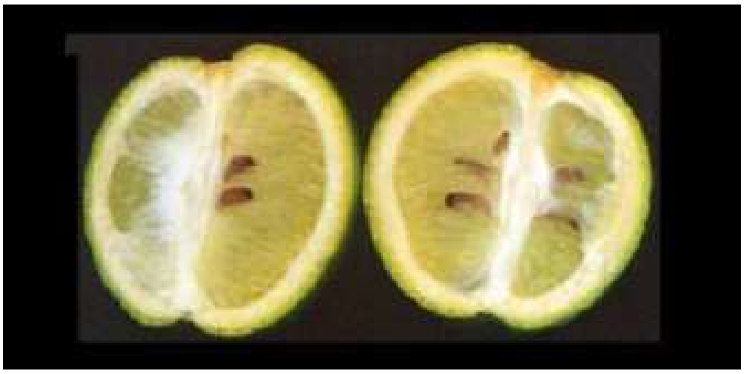


Image 7.- **Symptoms of HLB in fruits: deformed fruits and aborted seeds**

1.3.3.- Host plants

HLB host plants include several wild and cultivated rutaceae:

a) Citrus genus: all cultivated species and varieties are considered as susceptible to HLB, since the bacteria can persist and multiply in them, although the existence of different tolerance levels has been detected:

- Very sensitive: sweet orange, mandarin, grapefruit and tangelo,
- Sensitive: lemon, sweet lime, citron, kumquat and rough lemon,
- A little sensitive: Mexican lime and grapefruit,
- Relatively tolerant: *Poncirus trifoliata*

b) Ornamental rutaceae: jasmine orange (Murraya paniculata), Cape chestnut (Calodendrum capense), Clausena lansium, Severinia buxifolia.

Experimentally, by means of cuscuta, the disease has been transmitted to hosts of different plant families to the rutinaceae: the vinca of Madagascar (Catharanthus roseus) (Apocynaceae), tobacco (Nicotiana tabacum) and tomato (Lycopersicum esculentum) (Solanaceae).

1.4.- HLB control techniques

The best way to avoid losses due to citrus greening (HLB) is to avoid the entry and / or arrival of the disease.

In this sense, the risk that the disease arrives can be minimized by preventing the entry of plants and plant material infected by the bacteria (Candidatus Liberibacter spp.) Or by the psyllid vectors from infected areas. It is also important to regularly inspect the plantations and gardens in search of any symptoms of the psyllid vectors or HLB, raising awareness and training citizens in general, and agents of the citrus sector in particular, about the symptoms and the importance of giving notice to the competent authority of each country or region in case of any suspicion of its presence.

The chemical control of HLB vectors to reduce the incidence and spread of HLB is poorly compatible with European production systems subject to high levels of demand for residues in fruit ("zero waste") and existing regulations, which It is very restrictive in terms of the use of active materials.

The arrival of HLB would mean going from 2-3 treatments, which currently requires a citrus plantation per year, to more than 20 treatments that would be required for disease control. On the other hand, currently the European regulations do not include any authorized product for the control of the psila, requiring the development of more sustainable control strategies that integrate chemical, biological and technological measures.

Thus, the development of chemical control techniques, more rational and sustainable, classical biological control techniques, through the use of natural enemies of citrus psyllids, and technological techniques such as the control of non-productive sprouts (a in order to reduce psila populations) and the use of pattern-variety combinations, less attractive to psila and / or tolerant / resistant to HLB.

1.5.- Bibliography

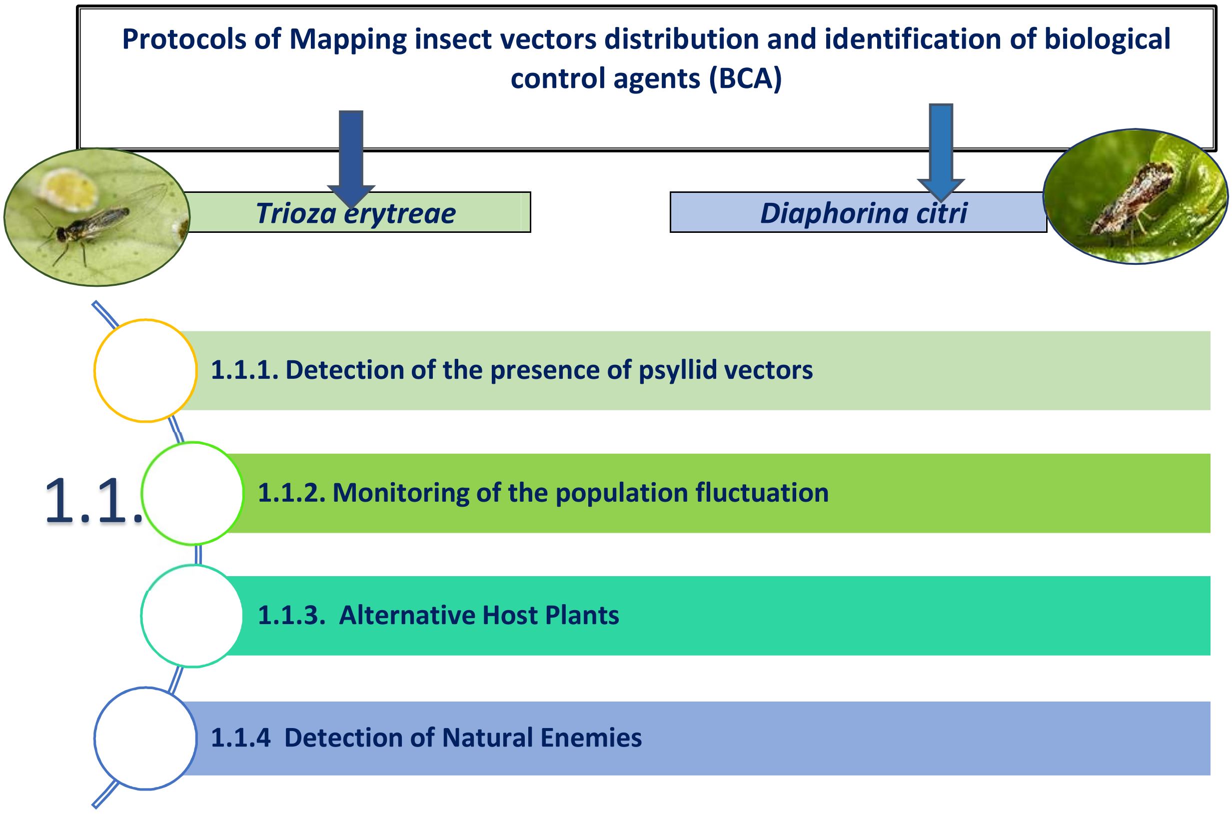
BOVÉ J.M. (2006). Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. *Journal of Plant Pathology*, 88 (1): 7-37.

PÉREZ-OTERO R., MANSILLA J.P., ESTAL P. (2015). Detección de la psila africana de los cítricos, *Trioza erytreae* (Del Guercio, 1918) (Hemiptera: Psylloidea: Triozidae), en la Península Ibérica. Arquivos Entomolóxicos, 13: 119-122.

DURÁN-VILA N., BOVÉ J.M. (2013). Huanglongbing es la enfermedad más devastadora de los cítricos. Levante Agrícola, 4º trimestre: 340-344.

2. Protocols of Mapping insect vectors distribution and identification of biological control agents (BCA)

Project title: Development of sustainable control strategies for citric under threat of climate change & preventing entry of HLB in EU



1.1. Protocols for mapping insect vectors distribution and identification of biological control agents (BCA)

This section has a double objective:

a) Present a protocol for early detection or monitoring of populations of insects vectors of Huanglongbing (HLB) of citrus fruits, *Trioza erytreae* (*Psylloidea, Triozidae*) and *Diaphorina citri* (*Psylloidea, Psyllidae*) psyllids, as a prevention tool against the possible arrival of disease vectors and basis of effective integrated management when they are present.
b) Present a guide of those organisms to conserve in our demonstration plots as potential natural enemies with the ability to regulate the populations of both species of psyllids.

It is of vital importance to prevent the appearance of the vector insect in new territories, and if it appears, determine its distribution, act quickly and efficiently, and combat it in order to eradicate it and prevent its spread. On the other hand, once established in a territory, it is necessary to know its population fluctuation and the natural factors that would allow us to regulate its populations, especially the generalist natural enemies present.

1.1.1. Detection of the presence of psyllid vectors

In order to detect the presence of the psyllid vectors of HLB in any area, it is essential to know how to identify the two species involved, as well as the different stages of these, at the same time that, the symptoms that both produce in the host plants. In the tables 1 and 2 are included the most relevant features, content to be expanded in the training package (Action A2)

<table>
<tr><th colspan="2">Table 1.- Symptoms of psyllid vectors of HLB in host plants</th></tr>
<tr><td colspan="2">Both T. erytreae and D. citri are considered minor citrus pests, but when their populations are high they can affect the vigor of the plant. The main damage caused by both insects is associated with the tree tender shoots, being able to interfere with the growth of plants, especially in young trees.</td></tr>
<tr><th>Trioza erytreae</th><th>Diaphorina citri</th></tr>
<tr><td></td><td></td></tr>
<tr><td>When nymphs feed on plant tissue, they produce gills in the form of concave bulges from the underside of it.
</td><td>Nymphs don’t protect themselves by forming gills and are mobile in all their stages.
</td></tr>
<tr><td>In very high populations the shoots are completely deformed and yellowish, but defoliation never occurs.
</td><td>Adults and nymphs cause twisting of the leaves and young shoots, and can reduce their elongation and, in some cases, the fall of the terminal leaves.</td></tr>
</table>

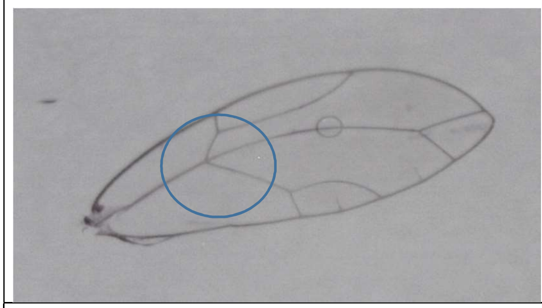
	
Both nymphs and adults excrete molasses that crystallizes and is deposited in the lower leaves, which favors the development of fumagina. 	Both nymphs and adults excrete abundant amounts of molasses that acquires a characteristic form of rolled filament. 

Table 2.- Differentiation of the developmental stages of the psyllid vectors of the HLB		
	Trioza erytreae	***Diaphorina citri***
ADULTS	Body length ≈ 4mm, initially light green color and then, acquire a dark tonality during maturity. Black head Almost completely black antennas, except the first segment that is whitish in appearance. Transparent wings, without spots, with a very marked venation.	Body length ≈ 3-4mm, light color with brown spots. Slightly brown head The antennas have black tips with two small light brown spots in the middle segments. Opaque wings of light shades, mottled with brown, with a wide longitudinal brown band characteristic.

		
	When they are feeding, adopt a very characteristic position, raising the body an angle of 35-45º with respect to the vegetal substrate.	
NYMPHS	Yellowed, flattened dorsiventrally and oval contour. Surrounded, even on the head, by a band of transparent waxy filaments. Advanced nymphs (N5) have two dark spots at the base of the abdomen. They are individually placed in galls. 	Initially light yellow, to later evolve towards more orange tones. The marginal waxy filaments are confined to the abdomen even in advanced nymphs (N5). More or less mobile in all its stages. They have no dark spots and are exposed and never produce galls. Clearly visible wing primordia. 
EGGS	Yellowed and oval shape with the pointed distal end. The eggs are laid with the long horizontal axis to the surface 	Almond-shaped, thicker at the base and narrows toward the distal end. Pale yellow freshly turned to orange near hatching. The eggs are placed in the plant tissue with the long vertical axis to the surface.  They are preferably placed on the stems of the terminal shoots.

There are some very marked differential aspects between these two species of psyllids, which could be explicitly taken into account for their differentiation:

Stage	*Trioza erytreae*	*Diaphorina citri*
Eggs	The eggs are laid with the long horizontal axis to the surface.	The eggs are placed in the plant tissue with the long vertical axis to the surface.
Nymphs	Nymphs develop on the leaves.	Nymphs develop on the stems of terminal shoots and petioles in an exposed manner.
	It produces molasses that crystallizes as a powder that is deposited on the plant substrate.	The nymphs excrete a waxy white substance as a thread that is deposited on the leaves.
	It produces galls open on the underside of the leaves in which the nymphs are placed individually. The galls are projected on the beam.	It does not produce galls. The nymphs are completely exposed and are more or less free to move
Adults	The angle of adults when feeding is about 35º, so that it is not as marked as in *D. citri.*	The adults, when feeding form an angle of 45º.
	Totally transparent wings. Main vein of the anterior wing trifurcated from the same point. 	Mottled brown wings. Main vein of the anterior wing bifurcated in two (media and cubitus). 
	Brownish color in the case of mature adults and light green in the case of newly emerged adults.	Light brown with dark brown spots.

They differ from aphids by the presence of legs with jumping structures, the absence of corniculas and the presence of winged eggs and males in all generations.

Symptoms of clementine leaf curl per citrus aphid

In the choice of a methodology that allows us to detect the presence of these psyllids in any area, a priori, the possibility of two situations should be taken into account: whether it is the time of tree sprouting or that the trees are in vegetative state, since this aspect will condition the method chosen later for detection and sampling.

Both species of psyllids are closely related to the presence of new shoots, since the eggs are laid exclusively on them, and the nymphs only develop on the young tissues of the host plant. Consequently**, if there is young sprouting in the trees, psyllids can be easily detected by observing the presence / absence of symptoms in the new sprouts**, and it would be confirmed by observing if the eggs or immature stages of these insects are present (Sétamou et al., 2008). Therefore, **the appropriate time to look for symptoms** is that in which there is sprouting, which covers almost the whole year, since citrus fruits generally follow an annual cycle in which four phases are distinguished:

- From the end of February to the beginning of May, spring sprouting takes place.
- Between July and August, summer sprouting takes place.
- From September to the end of November the third sprouting or autumn sprouting takes place.
- During winter they do not enter completely at rest, but reduce their vegetative activity.

The visual inspection would consist of selecting young shoots, looking for the presence of symptoms (table 1) or the presence of immature stages of these species (table 2). Species like lemon have more or less continuous flowering throughout the year, so it is the most susceptible species.
However, **if the tree is in a vegetative stage we must prospect to look for adults that may be resting in the foliage.** Therefore, during the winter or whenever the trees are in a vegetative stage, the presence and abundance of these psyllids can be determined by adhesive traps (Aubert & Hua, 1990; Hall et al., 2010; Sétamou et al., 2014; Monzó et al., 2015), insect vacuums for sampling (Thomas, 2012) and stem-tap samples (Stansly et al., 2010; Monzó et al., 2015).

Yellow Sticky Traps

They are yellow plastic cards with adhesive on both sides. It is recommended to use a minimum size of 12 x 20 cm, which offers a useful area of capture of 480 cm^2. They can be purchased commercially and trimmed to the required size. Yellow traps, are recommended for both psyllids, compared to other colors such as blue (Hall et al., 2007; Hall et al., 2010).

They are placed in the tree's sprouting area, approximately at the height of the operator's head (1.5 m high), and should be carefully checked in the laboratory to detect the presence of any of the psyllid species.

Stem-tap samples.

Stem-tap samples consists of hitting (manual beating) a randomly selected branch three times with a PVC tube (approx. 40 cm long and 2 cm in diameter). Adult psyllids are counted while falling on a splint with a 22 x 28 cm white sheet (approx. A4 size) or a white tray located horizontally below the branch.

Insect collection by suction

It involves the use of portable, lightweight and compact electrical devices with suction capacity that are modified for insect collection without damaging them. They are designed for use in the field.

The choice of one or the other method will depend on the particular conditions of each area, whether there are specialized personnel, etc. In table 3, we include a comparison of the different methods to serve as a guide when making our choice.

Table 3. Comparison of the different methods of monitoring adult populations of psyllids in citrus fruits.

Method	Application	Advantages	Inconveniences
Yellow Sticky Traps	It is recommended for early detection and population fluctuation studies. Adults are attracted to color and get stuck on the sticky surface. Applicable for both species of psyllid vectors.	They can be used in trees of low height (<1m). Higher accuracy than other samples with low population densities (for example, when frequent insecticide applications are performed) (Miranda et al., 2017). They can be used to monitor large areas.	They require a greater effort of sampling on time (they require facilities with magnifying glasses for their revision) and money (they are expensive). They are not specific to these species and someone with training is needed for their review and taxonomic determination.
Stem-tap samples	This technique allows us to detect adults who are in the citrus foliage without looking for them. It is recommended when is necessary the collection of specimens that will be used for the detection of HLB disease by molecular methods.	The monitoring times are faster than in visual observation. It is cheaper than sampling with yellow traps.	It must be done by an experienced monitor who can identify the copies of the vectors before they fly. It cannot be used on trees of low height. It would be only effective for the detection of adults in areas with established infestations.
Visual sampling of shoot	It is the only technique with which it is possible to count eggs and nymphs found exclusively in the shoots.	The advantages of this technique are low cost and rapid evaluation.	It is slanted by the capacity of the observer and his experience in the identification of the different stages of development. It requires more monitoring time.

			Although adults can be found in shoots, visual sampling requires more patience, especially when sprouting is scarce, since they are usually located under the leaves.
Insect collection by suction of insects	It can be applied both in cultivation as green areas or in the collection on natural vegetation, hedges, etc.	It is the most sensitive method for the detection of adults of psyllids when populations are scarce and widely dispersed.	It consumes a lot of time and is expensive to acquire. Personnel trained in taxonomy capable of separating catches are needed.

Other aspects of the biology of both species to be taken into account in their early detection, whether the is performed an observation of symptoms or if the presence of adults is sought by stem-tap samples or by means of adhesive chromatic traps, are:

Aspect	*Trioza erytreae*	*Diaphorina citri*
Position in the crop plot	Larger populations generally appear in trees located at the edge of the plantation.	
Tree Position	Adults tend to stand on shaded shoots.	Adults do not seem to show preferences for a specific location.
	Adults do not show preference for a specific orientation.	

Although several investigations have been carried out to find pheromones or kairomonas for adults of *D. citri* (Aksenov et al., 2014; Coutinho-Abreu et al., 2014), there are still no effective semiochemicals to attract a number suitable for adults of *D. citri* in the field population. These studies have not been carried out in the case of *T. erytreae*.

1.1.1-a. Inspection procedures in Green Areas

Although *T. erytreae* has been described as a weak flyer (Catling, 1973), it can be a highly invasive insect (Samways & Manicom, 1983). Under normal conditions, *T. erytreae* is relatively immobile and disperses only weakly, but when conditions become adverse, it can show a rather different type of behavior, with great dispersal capacity to locate new egg laying points (Samway, 1987).

For this reason, prospections should be based on the possible routes of entry of vector insects, which makes it possible to optimize the human resources available for it. Possible routes of entry are, for example: the natural dispersion from areas with presence of the pest by air currents and movements of susceptible material (ornamental hosts of the psyllids).

With the first spring sprouting, the surveys for the detection of symptoms should be started (table 1), subsequently confirming the presence of eggs or nymphal stages of the insects (Table 2). In this case, the observations should be directed towards young and tender shoots, and start from the margins of the area to be sampled.

Should be taken into account, all potentially host plants of these insects, which have eminently ornamental use, of both the rutinaceae family and other alternative species of different orders and families (see table listing host plants in section 1.1.3).

Yellow adhesive traps are probably the most widely used alternative worldwide for monitoring these psyllids in citrus fruits, but would not be recommended in the case of green areas. As an alternative, stem-tap samples could be used in green areas.

Two branches can be struck per tree in 10 groups of random trees in areas of possible psyllid entry.

1.1.1-b. Crop inspection procedures

As we mentioned in the section on green areas, a general proposal for a protocol for prevention or early detection is that, in the spring should begin prospecting for the detection of symptoms and/or signs of the insect.

If the crop plot has plants of different ages, it is better to focus monitoring on young plants and then continue with adults. Likewise, if the crop plot has different varieties, monitoring should be concentrated on those that are in sprouting or are more susceptible such as lemon.

Visual sampling for the count of immature stages is almost impossible, but in both species of psyllids it has been possible to correlate the number of individuals per outbreak with the percentage of occupied shoots (binomial sampling or presence / absence). If we opt for visual inspection, it would be done in the same way as in the case of stem-tap, that is, 10 groups of 4 trees are randomly sampled per hectare (a zig zag route is the most appropriate) observing the shoots of two branches by tree in the face of the cup that is in the interline; or 10 shoots (1 shoot per tree) are inspected at 10 stops per plot, including 5 stops on the margin of the plot and 5 stops inside the plot.

In the case of crops, yellow traps could be installed to capture adults during the vegetative period. The yellow traps should be placed at the height of the canopy preferably in trees on the periphery of the plots (adults will colonize them before), and should be carefully checked, since at first glance the psyllids could be confused with other similar insects (aphids, example) that can potentially be captured in the same trap. As these are areas without the presence of the pest, the revision of the yellow traps can be performed less frequently (e.g. monthly).

In the case of opting for Stem-tap samples, 10 stops per plot could be made on a zigzag route that includes 5 trees on the edge and 5 trees inside the plot, and in each of them hitting on 10 trees (one branch by tree) (Stansly et al., 2010).

1.1.2. Monitoring of the population fluctuation

Regular monitoring of psyllids in citrus fruits, when the plague is already present in a territory, is essential to know both the relative abundance and the seasonal evolution of them, and thus, choose the best time for the adoption of control measures.

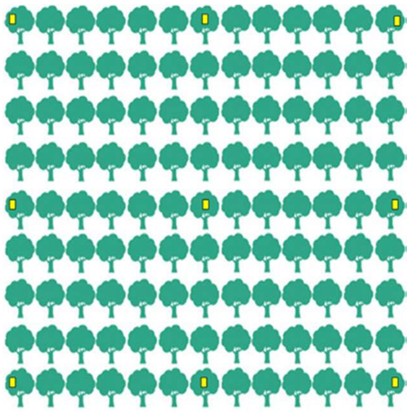
a) By means of Yellow Sticky Traps

Within each tree, the traps are placed in the periphery, at the height of the operator, in points of easy access so that the replacement of them is agile and fast. The traps are placed at an average distance of 100 meters between each trap. If the plot is smaller than 4 hectares, a minimum of 5 traps per plot will be placed, with a disposition of one trap per cardinal point and one in the center of it, regardless of its shape and size.

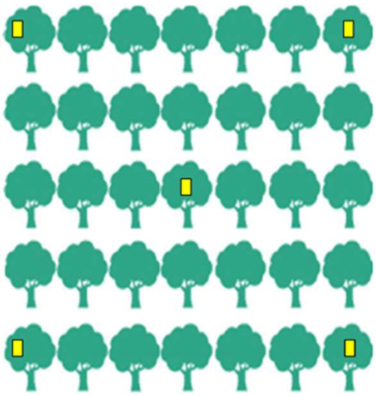
The change of traps should be carried out biweekly in the spring and summer months (in the presence of shoots) and monthly in the autumn and winter months (in the absence of shoots).

At the time of replacement, the trap is removed and protected with transparent film until the identification of the presence of insects in the laboratory; in this way, it prevents the damage to insects that are attached to the trap, which facilitates their correct identification.

When the traps cannot be processed on the same day of collection, they must be kept at 5°C for the conservation of the insects that remained attached until identification.



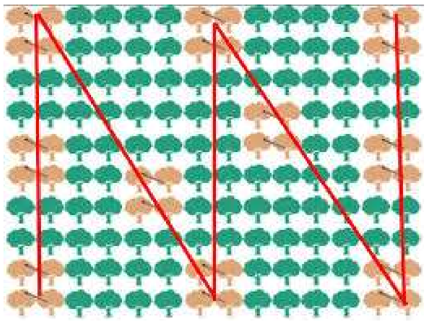
Trap placement design on plots larger than 4 ha



Design of placement of traps in plots smaller than 4 ha

b) By menas of Stem-tap samples

The sampling taken in Florida for the follow-up of *D. citri* includes 100 stem-tap distributed in 10 stops, in which 10 trees perstop (one branch per tree) are manual beating, preferably 5 trees on the edge and 5 on the inside of the plot (Stansly et al., 2010). The striking of 122 trees per plot has also been proposed in a zigzag route (Stansly, 2016; Monzo & Stansly, 2017). This method would also be useful for monitoring *T. erytreae.*



Another option, taken for the monitoring of *D. citri* in Brazil, is to select groups of 4 trees, from which two branches are manual beating per tree, in the face of the crown that is in the interlines and, 10 groups of four are monitored random trees per hectare, both outside and inside the plot following a zig-zag path.

1.1.3. Alternative Host Plants

Trioza erytreae is a plague of citrus fruits, although it can affect any species of the *Rutaceae* family, both wild (*Clausena anisata* (Willd.) Hook. F. Ex Benth, for example) and cultivated (CABI, 2014). It can feed on at least 18 plant species, but egg laying and nymphal development is restricted to 15 and 13 species respectively (Auber, 1987). Of citrus fruits, the lemon (*Citrus limon* (L.) Osbeck) and the lime (*Citrus aurantiifolia* (Christm.)) are the main guests (CABI, 2014). The potential host plants of *Trioza erytrea*e are those presented in Table 4.

In general terms, the host species of *Diaphorina citri* are the same mentioned as susceptible to *Trioza erytreae*. Additionally, high susceptibility has been observed in species other than Rutáceas, as in *Artocarpus* (Moraceae). In the US and Brazil, is mentioned to Rutácea, Murraya paniculata (Mirto or Limonaria or Jasmine), a plant that is characterized by a very strong smell of jasmine, as the most important host plant due to the susceptibility to both the vector and the bacterium. The potential host plants of *D. citri*, both from the rutinaceae family and other alternative orders and families, are those presented in Table 5.

Table 4. List of potential host plants of *Trioza erytreae* (African citrus psyllid)		
Rutaceae Family Scientific/Common Name	**Reference**	**Observations**
***Casimiroa edulis* La Llave ex Lex.** "Matasano, zapote blanco"	MAPAMA (2015)	**Synonyms:** -*Casimiroa edulis* f. microcarpa Martínez; *Zanthoxylum araliaceum* Turcz. It is not difficult to find it for sale in Spanish nurseries, especially in Andalusia, the Levantine area and the Balearic Islands.
***Choisya ternata* Kunth** "Mexican orange"	Cocuzza et al. (2017) Perez-Otero et al. (2015)	**Synonyms:** -*Choisya grandiflora* Regel -*Juliania caryophillata* La Llave & Lex.
Citrofortunella microcarpa (Bunge) Wijnands "calamondina o calamansí, naranjo enano"	MAPAMA (2015)	**Synonyms:** - *Citrus fortunella* - *Citrus mitis*
***Citroncirus webberi* J.W. Ingram & H.E. Moore.**	MAPAMA (2015)	**Synonyms:** Not found
Citrus aurantifolia (Christm.) Swingle	Cocuzza et al. (2017)	**Synonyms:** -*Citrus acida* Pers. -*Citrus davaoensis* (Wester) Yu.Tanaka -*Citrus depressa* var. voangasay (Bojer) Bory -*Citrus excelsa* Wester -*Citrus javanica* Blume -*Citrus lima* Lunan -*Citrus macrophylla* Wester This plant is reported as preferred host
***Citrus limon* (L.) Burm. F**	Cocuzza et al. (2017)	**Synonyms *Citrus limon* (L.) Burm. F:** -*Citrus limonum* Risso -*Citrus medica* subsp. *limonum* (Risso) -*Citrus medica* var. *limonum* (Risso) Lilja -*Citrus medica* var. *limon* L This plant is reported as common host
***Citrus maxima* (Burm.) Merr**	Cocuzza et al. (2017)	**Synonyms:** -*Aurantium corniculatum* Mill. -*Aurantium decumana* (L.) Mill. -*Aurantium distortum* Mill. -Aurantium maximum Burm. -*Citrus aurantium* var. crassa Risso -*Citrus aurantium* var. daidai Makino -*Citrus aurantium* var. decumana L. -*Citrus costata* Raf. -*Citrus decumana* L. nom. illeg. -*Citrus grandis* -*Citrus humilis* (Mill.) Poir. -*Citrus sinensis* This plant is reported as common host
***Citrus medica* L.**	Cocuzza et al. (2017)	**Synonyms:**

		-*Aurantium medicum* (L.) M. Gómez -*Citreum vulgare* Tourn. ex Mill. -*Citrus alata* (Tanaka) Yu.Tanaka -*Citrus aurantium* var. bergamia (Risso) Brandis -*Citrus aurantium* var. proper Guillaumin -*Citrus balotina* Poit. & Turpin -*Citrus bergamia* subsp. mellarosa (Risso) Rivera, et al. -*Citrus bicolor* Poit. & Turpin -*Citrus bigena* Poit. & Turpin -*Citrus cedra* Link -*Citrus tuberosa* Mill. -*Limon racemosum* Mill. -*Limon spinosum* Mill. This plant is reported as common host
Citrus paradisi **Macfad.**	Cocuzza et al. (2017)	**Synonyms:** -*Citrus Grandis* (Grapefruit) This plant is reported as common host
Citrus reticulata **Blanco** "Mandarino"	Cocuzza et al. (2017)	**Synonyms:** -*Citrus aurantium* f. deliciosa (Ten.) M.Hiroe -*Citrus aurantium* var. tachibana Makino -*Citrus aurantium* var. tachibana Makino -*Citrus chrysocarpa* Lush. This plant is reported as common host
Citrus sinensis (L.) **Osbeck**	Cocuzza et al. (2017)	**Synonyms:** -*Citrus aurantium* ß *sinensis* L. This plant is reported as common host
Citrus unshiu **(Swingle)**	Cocuzza et al. (2017)	**Synonyms:** Not found This plant is reported as common host
Clausena anisata **(Willd.)**	**Halbert & Manjunath (2004)** Cocuzza et al. (2017)	**Synonyms:** - *Amyris anisata* Willd. - *Amyris dentata* Willd. - *Amyris inaequalis* (DC.) Spreng. - *Amyris nana* Roxb. - *Amyris suffruticosa* Roxb. - *Clausena anisata var. mollis* Engl. - *Clausena anisata var. multijuga* Welw. ex Hiern - *Clausena anisata var. paucijuga* (Kurz) Molino - *Clausena bergeyckiana* De Wild. & T. Durand - *Clausena dentata* (Willd.) M.Roem. - *Clausena dentata var. dunniana* (H. Lév.) Swingle - *Clausena willdenowii* Wight & Arn. (illegitimate) - *Cookia dulcis* Bedd. - *Elaphrium inaequale* DC. - *Fagarastrum anisatum* G. Don - *Myaris inaequalis* (DC.) C. Pres This plant is reported as alternative host plant
Fagara **spp.**	**Halbert & Manjunath (2004)** Cocuzza et al. (2017)	**Synonyms:** -*Ochroxylum Schreb.* -*Xanthoxylum* Mill., orth. var.

		This plant is reported as alternative host plant
***Fortunella* spp.** **Species:** -*Fortunella crassifolia* -*Fortunella hindsii* -*Fortunella japonica* -*Fortunella margarita* -*Fortunella obovata* -*Fortunella polyandra* "kumquat"	Cocuzza et al. (2017) MAPAMA (2015)	**Synonyms:** Not found This plant is reported as alternative host plant
***Murraya exotica L. [=paniculata* (L.) Jack]**	Cocuzza et al. (2017)	**Synonym:** -*Chalcas* sp. -*Limonia malliculensis* This plant is reported as alternative host plant. It is a preferential host of *D. citri.*
***Murraya koenigii (L.)* Sprengel.** "Arbol de Curry"	MAPAMA (2015)	**Synonyms:** -*Bergera koenigii* L. 1771 -*Chalcas koenigii* (L.) Kurz 1875
***Poncirus trifoliata* (L.) Raf.**	Cocuzza et al. (2017)	**Synonyms:** -*Aegle sepiaria* DC -*Citrus trifolia* Thunb. -*Citrus trifoliata* L. -*Citrus trifoliata* subf. Monstrosa -*Citrus triptera* -*Limonia trichocarpa* -*Poncirus trifoliata* var. Monstrosa='Monstrosa' -*Pseudaegle sepiaria* This plant is reported as alternative host plant
***Toddalia asiatica* (L.) Lam.**	Cocuzza et al. (2017)	**Synonym:** - *Paullinia asiatica* This plant is reported as alternative host plant
***Vepris undulata* (Thunb.) Verdoorn & C.A. Smith** "White ironwood"	**Halbert & Manjunath (2004)** Cocuzza et al. (2017)	**Synonyms:** Not found This plant is reported as alternative host plant
***Vepris lanceolata* (Lam.) G. Don**	**Halbert & Manjunath (2004)** Cocuzza et al. (2017)	**Synonyms:** -*Toddalia lanceolata Lam* It is suggested that *T. erytreae* originally developed on this indigenous Rutaceae.
***Zanthoxylum* capensis (Thunb.) Harv.** "Small knobwood"	Cocuzza et al. (2017)	**Synonyms:** -*Fagara capensis* -*Fagara magaliesmontana* Engl. -*Zanthoxylum thunbergii* var. obtusifolia Harv. This plant is reported as alternative host plant
Alternative host plants	**Source/**	**Observations**

(Not Rutaceae family)	Reference	
Araliaceae family	**Cocuzza et al. (2017)**	Refers to the bibliographic citation of Hollis (1984)
Menispermaceae family	**Cocuzza et al. (2017)**	Refers to the bibliographic citation of Hollis (1984)

Tabla 5. List of potential host plants of vector *Diaphorina citri* (Asian citrus psyllid)		
Rutaceae Family Scientific/Common Name	**Reference**	**Observations**
Aegle marmelos (L.) Corr.	**Halbert & Manjunath (2004)** refers to the bibliographic citation of Viraktamath & Bhumannavar (2002)	**Synonyms:** -*Belou marmelos* (L.) A. Lyons -*Crateva marmelos* L.
Aeglopsis chevalieri Swingle	**Halbert & Manjunath (2004)** refers to the bibliographic citation of Koizumi et al. (1996)	**Synonyms:** -*Aeglopsis mangenotii* -*Aeglopsis beguei*
Afraegle **spp.** **Species:** *A. gabonensis Engl.* *A. paniculata (Schaum.) Engl.)*	**Halbert & Manjunath (2004)** refers to the bibliographic citation of DPI Citrus Arboretum surveys	**Synonyms:** ***Afragaele paniculata:*** **-** *Citrus paniculata,* **-** *Balsamocitrus paniculata.*
Amyris madrensis **S. Wats.**	**MAPAMA (2015)**	**Synonyms:** Is not found
Atalantia sp. *(Atalantia missionis Oliver, Atalantia monophylla (L.) Corr.)*	**Halbert & Manjunath (2004)** refers to the bibliographic citation of Koizumi et al. (1996) and Aubert (1990a)	**Synonyms:** -*Trichilia spinosa* Willd. -*Sclerostylis spinosa* (Willd.) Bl. -*Merope spinosa* M. Roem. -*Malnerega malabarica* Rafin. -*Limonia spinosa* Spreng. -*Limonia monophylla* Roxb. -*Atalantia spinosa* (Willd.) Hook. ex Koorders -*Atalantia puberula* Miq. In ***Atalantia sp.*** only adult insect feeding was detected.
Balsamocitrus dawei Stapf	**MAPAMA (2015)**	**Synonyms:** Is not found
Choisya Kunth **Species:** *Choisya arizonica* *Choisya dewitteana* *Choisya dumosa* *Choisya grandiflora* *Choisya katherinae* *Choisya ternata* Kunth - naranjo mexicano	**MAPAMA (2015)**	**Synonyms**: *Astrophyllum*.

***Citropsis schweinfurthii (Engl.)* Swingle & Kellerm.**	**Halbert & Manjunath (2004)** refers to the bibliographic citation of Chavan & Summanwar (1993)	**Synonyms:** -*Citropsis mirabilis* (A. Chev.) Swingle & M. Kellerm. -*Citropsis preussii* (Engl.) Swingle & M. Kellerm. -*Citrus articulata* Willd. ex Spreng. -*Limonia mirabilis* A. Chev. -*Limonia preussii* This plant is reported as a good host for ***D. citri***
Citrus aurantifolia (Christm.) Swingle	**Halbert & Manjunath (2004)** refers to the bibliographic citation of Aubert (1987, 1990ª), Florida surveys.	**Synonyms:** -*Citrus acida* Pers. -*Citrus davaoensis* (Wester) Yu. Tanaka -*Citrus depressa* var. voangasay (Bojer) Bory -*Citrus excelsa* Wester -*Citrus javanica* Blume -*Citrus lima* Lunan -*Citrus macrophylla* Wester This plant is reported as preferred host for ***D. citri***
Citrus aurantium	**-Tsai, J.H & Liu, Y.J. (2000)** **-Halbert & Manjunath (2004)** refers to the bibliographic citation of Florida surveys.	**Synonyms:** -*Aurantium* sp. (*Aurantium acre* Mill., -*Aurantium bigarella* Poit. & Turpin, -*Aurantium corniculatum* Mill., etc) -*Citrus* sp. (*Citrus bigaradia* Risso & Poit., *Citrus humilis* (Mill.) Poir., *Citrus amara* Link, *Citrus aurata* Risso, etc)
***Citrus deliciosa* Tenore**	**Halbert & Manjunath (2004)** refers to the bibliographic citation of Aubert (1987)	**Synonyms:** -*Citrus aurantium* f. deliciosa (Ten.) M.Hiroe -*Citrus aurantium* var. tachibana Makino -*Citrus aurantium* var. tachibana Makino -*Citrus chrysocarpa* Lush. This plant is reported as common host of ***D. citri.***
***Citrus jambhiri* Lush.**	**-Tsai, J.H & Liu, Y.J. (2000)** **-Étienne, et al. (2001)** **- Halbert & Manjunath (2004)** refers to the bibliographic citation of Florida surveys.	**Synonyms:** Is not found
***Citrus japonica* var. madurensis (Loureiro) Guillaum (Calamondín)**	**- Rae, *et al.* (1997)** **- Halbert & Manjunath (2004)**	**Synonyms:** -*Atalantia hindsii* -*Atalantia monophylla Benth. -Citrofortunella madurensis* (Lour.) -*Citrus aurantium* var. Japónica -*Fortunella* sp. -*Sclerostylis hindsii* Champ. ex Benth -*Sclerostylis venosa Champ*. ex Benth
***Citrus limon* (L.) Burm. F**	**Halbert & Manjunath (2004)** refers to the bibliographic citation of Aubert (1987, 1990a) **Nava et al. (2007)**	**Synonyms:** -*Citrus limonum* Risso -*Citrus medica* subsp. *limonum* (Risso) -*Citrus medica* var. *limonum* (Risso) Lilja -*Citrus medica* var. *limon* L This plant is reported as common host of ***D. citri.***
***Citrus maxima* (Burm.) Merr**	**Halbert & Manjunath (2004)** refers to the	**Synonyms:** -*Aurantium corniculatum* Mill.

	bibliographic citation of Aubert (1990a)	-*Aurantium decumana* (L.) Mill. -*Aurantium distortum* Mill. -Aurantium maximum Burm. -*Citrus aurantium* var. crassa Risso -*Citrus aurantium* var. daidai Makino -*Citrus aurantium* var. decumana L. -*Citrus costata* Raf. -*Citrus decumana* L. nom. illeg. -*Citrus grandis* -*Citrus humilis* (Mill.) Poir. -*Citrus sinensis* This plant is reported as occasional host, but observed nymphal development of ***D. citri***.
Citrus medica L	**Halbert & Manjunath (2004)** refers to the bibliographic citation of Aubert (1987, 1990a)	**Synonyms:** -*Aurantium medicum* (L.) M. Gómez -*Citreum vulgare* Tourn. ex Mill. -*Citrus alata* (Tanaka) Yu. Tanaka -*Citrus aurantium* var. bergamia (Risso) Brandis -*Citrus aurantium* var. proper Guillaumin -*Citrus balotina* Poit. & Turpin -*Citrus bergamia* subsp. mellarosa (Risso) Rivera, et al. -*Citrus bicolor* Poit. & Turpin -*Citrus bigena* Poit. & Turpin -*Citrus cedra* Link -*Citrus tuberosa* Mill. -*Limon racemosum* Mill. -*Limon spinosum* Mill. This plant is reported as common host of ***D. citri.***
Citrus nobilis **Lour**	**Halbert & Manjunath (2004)** refers to the bibliographic citation of Aubert (1987) and Florida surveys	**Synonyms:** -*Citrus aurantium* f. deliciosa (Ten.) M.Hiroe -*Citrus aurantium* var. tachibana Makino -*Citrus aurantium* var. tachibana Makino -*Citrus chrysocarpa* Lush. This plant is reported as common host of ***D. citri.***
Citrus paradisi **Macfad.**	**Halbert & Manjunath (2004)** refers to the bibliographic citation of Aubert (1987), Florida surveys and Tsai & Liu (2000)	**Synonyms:** -*Citrus Grandis* (Grapefruit) This plant is reported as host of ***D. citri***: common host, a preferred host in Florida and (DPI), best host in laboratory assays (Tsai & Liu, 2000).
Citrus sinensis (L.) **Osbeck**	**Halbert & Manjunath (2004)** refers to the bibliographic citation of Aubert (1987, 1990a) and Florida surveys	**Synonyms:** -*Citrus aurantium* ß *sinensis* L. This plant is reported as common host of ***D. citri.***
Citrus **spp.**	**Halbert & Manjunath (2004)** refers to the bibliographic citation of Aubert (1990a) and Florida surveys	**Synonyms:** -*Afraurantium* A. Chev. -*Aurantium* Mill. -*Citrofortunella* J.W. Ingram & H.E. Moore -*Clymenia Swingle* -*Eremocitrus Swingle* -*Feroniella Swingle* -*Fortunella Swingle* -*Microcitrus Swingle*

		Theses plants are reported as common host of ***D. citri.***
Clausena anisum-olens **Merrill**	**Halbert & Manjunath (2004)** refers to the bibliographic citation of Aubert (1990a)	**Synonyms:** -*Cookia anisum-olens* Blanco -*Clausena laxiflora* Quis. & Merrill -*Clausena sanki* (Perr.) Molino This plant is reported as occasional host of ***D. citri,*** observed nymphal development.
Clausena lansium **(Lour.) Skeels**	**Halbert & Manjunath (2004)** refers to the bibliographic citation of Koizumi et al. (1996), Aubert (1990) and Florida surveys	**Synonyms:** -*Clausena wampi* (Blanco), Oliver. -*Clausena punctata* (Sonn.), Rehd. & E.H. Wils This plant is reported as host of ***D. citri:*** poor host (Koizumi et al., 1996), common host (Aubert, 1990), population highly variable (FL surveys).
Eremocitrus glauca **(Lindl.) (Australian desert lime)**	**MAPAMA (2015)**	**Synonym:** -*Triphasia glauca* Lindl.
Esenbeckia berlandieri **Baill. ex Hemsl**	**MAPAMA (2015)**	**Synonyms:** -*Esenbeckia berlandieri* subsp. berlandieri -*Esenbeckia ovata* Brandegee -*Esenbeckia yaaxhokob* Lundell
Fortunella **spp.**	**Halbert & Manjunath (2004)** refers to the bibliographic citation of Aubert (1987, 1990a)	**Species:** -*Fortunella crassifolia* -*Fortunella hindsii* -*Fortunella japonica* -*Fortunella margarita* -*Fortunella obovata* -*Fortunella polyandra* These plants are reported as occasional host of ***D. citri***, nymphal development in laboratory only (Aubert 1990).
Glycosmis pentaphylla	**MAPAMA (2015)**	**Synonyms:** - *Bursera nitida* Fern.-Vill. - *Chionotria monogyna* Walp. - *C. rigida* Jack - *Glycosmis arborea* (Roxb.) DC. - *G. arborea* var. linearifoliolata V. Naray. - *G. chylocarpa* Wight & Arn. - *G. madagascariensis* Corrêa ex Risso - *G. pentaphylla* (Retz.) Corrêa - *G. pentaphylla* var. linearifoliolis Tanaka - *G. quinquefolia* Griff.
Microcitrus Swingle	**MAPAMA (2015)**	**Synonym:** -*Microcitrus australasica* (F. Muell.) Swingle 1915
Murraya koenigii **(L.) Sprengel**	**Halbert & Manjunath (2004)** refers to the bibliographic citation of Koizumi et al. (1996), Aubert (1987, 1990ª), Lim et al. (1990) and Florida surveys	**Synonyms:** -*Bergera koenigii* L. -*Chalcas koenigii* (L.) Kurz This plant is reported as host30 of ***D. citri:*** good host (Koizumi, 1996), occasional host, no eggs observed (Aubert, 1987), good host with nymphal development (Aubert, 1990a), not an excellent host but will support a small population, including eggs (FL surveys)

***Murraya paniculata* (L.)**	**- Rae, *et al.* (1997)** **- Étienne, et al. (2001)** **- Tsai, J.H & Liu, Y.J. (2000)** **- Halbert & Manjunath (2004)** refers to the bibliographic citation of Koizumi et al. (1996), Aubert (1987) and Florida surveys **Nava et al. (2007)** **-Lozano-Contreras & Ramírez-Jaramillo (2018)**	**Synonyms:** -*Murraea exotica* L. -*Connarus* sp. -*Chalcas* sp. -*Limonia malliculensis* It is a preferential host of *D. citri.* It blooms throughout the year, its flowers are fragrant. It is also a host plant for predators of the *Coccinellidae* and *Chrysopidae* families, as well as the parasitoid *Tamarixia radiata* (Hymenoptera). This plant is widely planted in urban areas as landscape hedge, it could serve as an alternate host for maintaining high *D. citri* populations during the periods when young citrus shoots are not available. Murraya spp. are good hosts of *D. citri*, and are resistant or at least tolerant to citrus greening disease.
***Naringi crenulata* (Roxb.) Nicolson**	**MAPAMA (2015)**	**Synonyms:** -*Hesperethusa crenulata* (Roxb.) Roem. -*Limonia crenulata* Roxb.
Pamburus missionis (Wall. ex Wight) Swingle	**MAPAMA (2015)**	**Synonym:** -*Limonia missionis* Wight
***Poncirus trifoliata* (L.) Raf.**	**Halbert & Manjunath (2004)** refers to the bibliographic citation of Koizumi et al. (1996), Aubert (1987, 1990a)	**Synonyms:** -*Aegle sepiaria* DC -*Citrus trifolia* Thunb. -*Citrus trifoliata* L. -*Citrus trifoliata* subf. Monstrosa -*Citrus triptera* -*Limonia trichocarpa* -*Pseudaegle sepiaria* This plant is reported as occasional host of ***D. citri***, eggs, but no nymphs (Aubert 1987, 1990a)
***Severinia buxifolia* (Poir.) Ten.**	**MAPAMA (2015)**	**Synonyms:** -*Atalantia bilocularis* (Roxb.) Wall. ex Skeels -*Atalantia buxifolia* (Poir.) Oliv. -*Citrus buxifolia* Poir. -*Limonia bilocularis* Roxb. -*Sclerostylis buxifolia* (Poir.) Benth.
***Swinglea glutinosa* (Blanco) Merr.**	**MAPAMA (2015)**	**Synonyms:** -*Limonia glutinosa* Blanco
***Tetradium ruticarpum* (A. Juss) T.G. Hartley**	**MAPAMA (2015)**	**Synonyms:** -*Euodia ruticarpa* (A. Juss.) Benth. -*Evodia ruticarpa* (A. Juss.) Hook.f. & Thomson
***Toddalia asiática* (L.) Lam**	**Halbert & Manjunath (2004)** refers to the bibliographic citation of Aubert (1987, 1990a)	**Synonyms:** -*Toddalia aculeata* Pers. -*Toddalia ambigua* Turcz. -*Toddalia angustifolia* Lam. -*Toddalia asiatica gracilis* Gamble -*Toddalia asiatica* var. floribunda (Wallich) Kurz -*Toddalia asiatica* var. obtusifolia Gamble -*Toddalia effusa* Turcz. -*Toddalia floribunda* Wall. -*Toddalia micrantha* Steud.

		-*Toddalia nitida* Lam. -*Toddalia rubicaulis* Roem. & Schult. -*Toddalia schmidelioides* Baker -*Toddalia tonkinensis* Guill. -*Toddalia willdenowii* Steud. **Common names:** orange climber (Eng), ranklemoentjie (Afr), gwambadzi (Venda) This plant is reported as occasional host of ***D. citri***, no eggs observed.
***Triphasia trifolia* (Burm. f.) P. Wilson**	**Halbert & Manjunath (2004)** refers to the bibliographic citation of Koizumi et al. (1996), Aubert (1987, 1990a) and DPI Citrus Arboretum survey	**Synonyms:** -*Limonia trifolia* Burm. f., -*Triphasia aurantiola* Lour. This plant is reported as poor host (Koizumi, 1996), occasional host (Aubert, 1987), all stages and damage evident (FL surveys).
Alternative host plants (Not Rutaceae family)	**Source/ Reference**	**Observations**
***Artocarpus heterophyllus* Lamarck**	**Halbert & Manjunath (2004)** refers to the bibliographic citation of **Shivankar et al. (2000)**	**Synonyms:** -*Artocarpus brasiliensis* Ortega -*Artocarpus maximus* Blanco -*Artocarpus nanca* Noronha -*Artocarpus philippensis* Lam.

1.1.4. Detection of Natural Enemies

In this section we simply list those natural enemies of interest in the biological control of the two species of psyllid HLB vectors. Information on refuge plants for these species or those species of greatest interest will be expanded in section 3.2 on control methods.

1.1.4-a) Natural enemies of *Trioza erytreae*

Predators:

According to Catling (1970) field studies carried out in Transval and in Swaziland, they showed that ***T. erytreae*** is attacked by a complex of predators, including several species of neuroptera (crisopa, hemerobiidae and coniopterygidae), diptera (syrup), coleoptera (coccinellids), spiders and mites. Also in Madeira, they were able to observe that the populations of these predators, although little synchronized with ***T. erytreae*** at the beginning of summer, contributed to the reduction of the pest from mid-summer, continuing quite active in autumn and early winter (Passos from Caevalho & Franquinho Aguiar, 1997). The neuroptero *Chrysopa pudica* (Navás, 1914), the Syrphus *Allograpta pfeifferi* (Bigot, 1884), the coccinellid *Cheilomenes propinqua* (Mulsant, 1850), and the phytoseido *Iphiseius degenerans* (Berlese, 1889) are cited.

Estévez et al. (2018) in their study on the natural biological control of the African psyllid in the citrus crops of the Canary Islands, they observed ten predatory species (a neuroptera, two anthocorrids, six coccinellids, one syrphus). These authors report the Neoptera *Chrysoperla carnea* (Stephens), the sphynx *Episyrphus balteatus* (De Geer), the coccinellids *Harmonia axyridis* Pallas, *Exochomus quadripustulatus* (L.), *Olla v-nigrum* (Mulsant), *Cryptolaemus montrouzieri* Mulsant, *Delphastus catalinae* (Horn) and the anthocorid hemiptera *Orius albidipennis* Reuter (as = *O. laevigatus*) and *Anthocoris* sp.

Parasites:

Catling (1969b) showed in his studies, that parasitism is an important factor in the control of psila populations, the intensity of the parasitism being variable, depending mainly on the synchronization between the parasitoid and psila populations. In his studies the main parasite found was ***Tamarixia dryi*** (=***Tetrastichus radiadus*** Waterston) (Waterston, 1922), sometimes surpassed by ***Psyllaephagus pulvinatus*** (Waterston).

McDaniel & Moran (1972) also refer to the importance of parasitoids as limiting factors for the abundance of the nymph populations of ***T. erytreae***, mainly the eulophid ***T. dryi***, which acts as an ectoparasite, and the encycled endoparasite ***Psyllaephagus pulvinatus*** (Waterston). In the same way that Catling (1969), these authors shows the importance for the control of the pest, of the relationship between parasitoids and their hyperparasitoids, mostly ***Aphidencyrtus cassatus***, encyrtid present in the Rhodesian región, described for the first time by Annecke (1969).

Prinsloo (1980) establishes that the species next to ***T. radiatus***, often referred to as ***T.? radiatus***, in much of the bibliography on the biological control of ***T. erytreae*** is actually the eulophid ***Tamarixia dryi***, native species of Africa and main parasitoid of ***T. erytreae*** (Prinsloo, 1984). Some authors have commented on the existence of another eulophid ectoparasite, ***Tetrastichus sicarius*** Waterston, which, like ***T. dryi***, attacks the nymphal stages 3, 4 and 5 of ***Trioza erytreae***, and both originally have been described in Kenya. However, some taxonomists consider ***T. sicarius*** as a synonym for ***T. dryi***.

Tamesse (2009) in his study in Cameroon, cites the following parasites: ***Syrphophagus cassatus***, ***Psyllaephagus pulvinatus, Tamarixia*** sp. and ***Tamarixia dryi***. Several species like ***Dilyta* sp**. (*Figitidae)* and ***Aphanogmus* sp.** *(Ceraphronidae),* were cited for the first time in this study, in the parasitoid complex of ***T. erytreae***.

In the particular case of Reunion Island, in 1972, was carried out the introduction of ***Tamarixia dryi*** and ***Psyllaephagus pulvinatus***. Between 1972 and 1979, ***T. dryi*** was fully established, greatly reducing the number of psyllids in citrus fruits. In 1974, two years after the release of ***T. dryi***, a significant reduction in attacks of ***T. erytreae*** was observed in areas of the island above 500 meters above sea level. In 1976, only a few shoots of lemon trees had symptoms of galls on the leaves, and since 1979, ***T. erytreae*** detections were very low, less than 5 leaves per lemon tree with symptoms throughout the island. Since 1980, due to the high percentage of parasitism obtained after the releases of ***T. dryi*** (Aubert et al., 1980) the pest was considered eradicated.

Taking into account the results in Reunion Island, it has been demonstrated that in biological control experiences of ***T. erytreae***, the best results have been obtained through classical biological control, with the introduction of the specific parasitoid ***Tamarixia dryi***.

Estevez, et al. (2018) did not observe parasitism by hymenoptera, nor evidence of this on any of the nymphal stages of ***T. erytreae***. They only observed very frequently the presence of larvae of a mite belonging to the family ***Erythraeidae* (*Trombidiformes, Prostigmata*);** the larvae of these organisms are parasites of other arthropods, while adults are free-living predators.

Pérez-Rodríguez et al. (2019) also showed that parasitoids of the genus *Tamarixia* are among the most effective natural enemies of ***T. erytreae*** in South Africa, especially ***Tamarixia dryi***. This species was the most abundant primary parasitoid (90-95%) followed by ***P. pulvinatus*** (79-80%) and ***Tamarixia* sp.** (65-70%). The most abundant hyperparasitoid was the infected ***Aphidencyrtus cassatus*** Annecke.

Entomophatogenics

In South Africa, the ***Cladosporium oxysporum*** fungus was isolated from the pseudococcal ***Planoccocus citri***, and was multiplied and tested in the laboratory against ***T. erytreae*** with good results (Samways & Grech, 1986). Likewise, it has been observed that ***Capnodium citri***, which induces a blackish mold, commonly known as fumagina or bold, is capable of causing a fungal epizootic in ***T. erytreae*** in artificial breeding. However, the effects of the two fungi are dependent on density, which is not compatible with the control of low levels of plague, being frequent on natural conditions (Aubert, 1987).

Table 6. Natural enemies of *Trioza erytreae*

Species	Type (Pred/Par/Entomo)*	Stage of Host	CSA*	Reference and location
Adalia bipunctata **(Coleoptera, Coccinellidae)**	**Predator of aphids, ocassional predator of psyllid.**	**Eggs**	**CA**	**Cocuzza et al. (2016)** Canary Island
Brumus quadripustulatus **(Coleoptera, Coccinellidae)**	**Predator of aphids**		**CA**	**Cocuzza et al. (2016)** Canary Island
Allograpta pfeifferi **(Diptera)**	**Predator** (only when they were the only food offered and after 1 - 2 days)	**Nymphs**	**CA**	**Hristova (2014)**
Cheilomenes propinqua **(Coleoptera)**	**Predator**	**Nymphs and adults**	**CA**	**Hristova (2014)**
Chilocorus nigritus **(F.) (Coleoptera, Coccinellidae)**	**Ocassional predator of psyllid**	**Nymphs**	**CA**	**Cocuzza et al. (2016)** South Africa
Chrysopa pudica **Nava (Neuroptera, Chrysopidae)**	**Generalist predators**	**Nymphs**	**CA**	**Cocuzza et al. (2016)** South Africa
Chrysoperla carnea **(Neuroptera, Chrysopidae)**	**Generalist predators**	**Nymphs and eggs**	**CA**	**Cocuzza et al. (2016)** Canary Island
Cladosporium oxysporum **(Berk. & Curt.)**	**Entomophatogenic fungus**	**Nymphs**	**CA**	**(Samways & Grech, 1986)** **Cocuzza et al. (2016)** South Africa
Cryptolaemus montrouzieri **(Coleoptera, Coccinellidae)**	**Predator of mealybugs, ocassional predator of psyllid.**	**Eggs**	**CA**	**Cocuzza et al. (2016)** Canary Island
Exochomus quadripustulatus (L) **(Coleoptera, Coccinellidae)**	**Predator of mealybugs, ocassional predator of psyllid.**	**Nymphs**	**CA**	**Estévez et al. (2018)** Canary Island
Episyrphus balteatus **(Diptera, Syrphidae)**	**Generalist predator**	**Nymphs**		**Hristova (2014)**
Delphastus catalinae (Horn) **(Coleoptera, Coccinellidae)**	**Predator of white flies and ocassional predator of psyllid.**	**Eggs**	**CA**	**Estévez et al. (2018)** Canary Island

Harmonia axyridis **(Coleoptera, Coccinellidae)**	**Predator**	**Nymphs and eggs**	**CA**	**Cocuzza et al. (2016)** Canary Island
Iphiseius degenerans (Berlese) **(Acari, Phytoseiid)**	**Predator of mites, ocassional predator of psyllid.**	**Nymphs and eggs**	**CA**	**Cocuzza et al. (2016)** **Hristova (2014)** South Africa
Leptus sp. **(Acari, Erythraeidae)**	**Parasite**	**Nymphs and adults**	**CA**	**Cocuzza et al. (2016)** Canary Island
Microtus sjoestedti **Wheel**	**Generalist predator**	**Nymphs**	**CA**	**Cocuzza et al. (2016)** South Africa
Mites belonging to the genera ***Bochartia*** and ***Abrolophus***	**Ocassional predator of psyllid.**	**Nymphs**	**CA**	**Cocuzza et al. (2016)** South Africa
Olla v-nigrum **(Mulsant) (Coleoptera, Coccinellidae)**	**Predator of coccidae, ocassional predator of psyllid.**	**Nymphs**	**CA**	**Estévez et al. (2018)** Canary Island
Orius albidipennis **(Hemiptera, Anthocoridae)**	**Generalist predator**	**Eggs**	**CA**	**Cocuzza et al. (2016)** Canary Island
Psyllaephagus pulvinatus **Waterston (Hymenoptera, Encyrtidae)**	**Parasitoide: Primary, Solitary, koinobiont endoparasitoid** Is frequently attacked by a complex of hyperparasitoids	**Nymphs**	**CA**	**Tamesse (2009)** **Cocuzza et al. (2016)** **Perez-Rodriguez et al. (2019)** South Africa, Swaziland and Cameroom
Syrphophagus cassatus **Annecke (Hymenoptera, Encyrtidae)**	**Parasitoide: secondary**			**Tamesse (2009)** Cameroom
Tamarixia dryi **(=*Tetrastichus dryi)* (Waterston) (Hymenoptera, Eulophidae)**	**Parasitoide: Primary, Solitary, koinobiont ectoparasitoid** Has been reported as the most common and effective parasitoid of *T. erytreae* Is frequently attacked by a complex of hyperparasitoids	**Nymphs**	**CA BCD**	**Tamesse (2009)** **Perez-Rodriguez et al. (2019)** Cameroom Canary Island, South Africa, Reunion Island, Mauritius, Swaziland
Tamarixia **sp. (Hymenoptera, Eulophidae)**	**Parasitoide: Primary parasitoid.**	**Nymphs**	**CA BCD**	**Perez-Rodriguez, J., Kruger, K., et al. (2019)** South Africa
Dilyta **sp. (Hymenoptera, Figitidae)**		**Nymphs**		**Tamesse, J.L. (2009)** Cameroom
Aphanogmus **sp. (Hymenoptera, Ceraphronidae)**		**Nymphs**		**Tamesse, J.L. (2009)** Cameroom

***Type of NE:** Predator (Pre), Parasite (Par), Entomophatogenic (Entomo)
***Current state of application of the natural enemy (CSA):** Research (Re), Cited application (CA), Biological control demonstrated
with results in the field (BCD)

1.1.4-b) Natural enemies of *Diaphorina citri*
Predators:

The species complex of biological control agents attacking ***D. citri*** varies geographically, but usually includes various species of ladybeetles (Coleoptera: Coccinellidae); syrphid flies (Diptera: Syrphidae); lacewings (Neuroptera: Chrysopidae, Hemerobiidae); and spiders (Aranae).

One species of ***Scymnus*** (Coccinellidae) has been reported in Brazil (Gravena et al. 1996). Syrphids in the genus ***Allograpta*** have been found in Reunion and Nepal (Aubert 1987) and in Florida (Michaud 2002). Miranda et al. (2008) describe the fallowing insects as predators of ***D. citri Cycloneda sanguinea*** (L.), ***Chilocorus cacti*** (L.), ***Exochomus cubensis*** Dimn, ***Scymnus distinctus*** Casey (Coleoptera: Coccinellidae), ***Chrysopa*** **sp**. (Neuroptera: Chrysopidae) y ***Ocyptamus*** **sp**. (Diptera: Syrphidae). In Florida, the most abundant predators are ***Harmonia axyridis*** and ***Olla v-nigrum*** (Michaud 2001; Michaud 2002; Michaud 2004). ***Olla v-nigrum*** it was a relatively rare species before the arrival of ***D. citri***, but exhibited a marked functional response to the establishment of ***D. citri*** (Michaud 2001).

Both Michaud (2002, 2004) in Florida and Al-Ghamdi (2000) in Saudi Arabia have observed that spiders may be important predators for ***D. citri.*** In Saudi Arabia, spiders accounted for 33.6% of total predators (Al-Ghamdi 2000). Several other predators, including a histerid beetle, *Saprinus chalcites* Illiger (Al-Ghamdi 2000).

Michaud & Olsen (2004) performed a series of experiments to assess the nutritional suitability of ***D. citri*** as a prey for 5 species of coccinellids. The results of the consumption assays reveal that adults of ***H. axyridis, C. coeruleus*** and ***O. v-nigrum*** are voracious feeders on ***D. citri***.

Parasites:

There are two well-known primary parasites of ***D. citri***: one is a eulophid ectoparasite, ***Tamarixia (=Tetrasticus) radiata*** **(Waterston)** (***Hymenoptera: Eulophidae***) and the other is an encyrtid endoparasite, ***Diaphorencyrtus aligarhensis*** (Shaffee et al., 1973) **(*Hymenoptera: Encyrtidae*)**. ***Tamarixia radiata*** is more efficient at parasitizing ***D. citri*** than ***D. aligarhensis*** (Tang, 1989).

Tamarixia radiata is an idiobionte specific ectoparasitoid of nymphs of the Asian citrus psyllid, which was described from specimens that emerged from parasitized nymphs of ***D. citri*** on lemon leaves collected in Pakistan (Waterston, 1922). ***T. radiata*** was successfully introduced for the control of ***D. citri*** in the Reunion Islands, Taiwan, Mauritius, the Philippines, Saudi Arabia, Indonesia (East Java), Guadeloupe and the United States (Florida) (Grafton-Cardwell et al., 2013).

Entomophatogenics

The pathogenic fungi may be the most important mortality factor for ***D. citri,*** nymphal mortality of 60-70% could be expected where minimum daily relative humidity is high (Aubert 1987). Other entomopathogenic fungi have been reported, these include ***Isaria fumosorosea*** (Wize) ***(=Paecilomyces fumosoroseus)*** (Hypocreales: Cordycipitaceae), ***Lecanicillium lecanii*** R. Zare & W Gams, ***Beauveria bassiana*** (Bals.) Vuill., e ***Hirsutella citriformis*** **Speare** (Subandiyah et al., 2000; Meyer et al. 2007; Humber et al., 2011; Casique-Valdes et al., 2011)

Table 7. Natural enemies of *Diaphorina citri*

Species	Type (Pred/Par/Entomo)*	Stage of Host	CSA*	Reference and location
***Anystis baccarum* (L.) (Acari)**	**Ocassional predator**		**CA**	**Yang et al. (2006)** cited in **Hall (2008)** China
***Beauveria bassiana* (Bals.) Vuill.**	**Entomophatogenic fungus**		**CA**	**Rivero Aragon & Grillo-Ravelo (2000)** **Yang et al. (2006)** cited in **Hall (2008)** Cuba, China
***Brumus suturalis* (Fab.) (Coleoptera: Coccinellidae)**	**Generalist predator**		**CA**	**Hall (2008)** India
***Capnodium citri* Mont.**	**Entomophatogenic fungus**	**Nymphs**	**CA**	**Aubert (1987)** cited in **Halbert & Manjunath (2004) y Hall (2008)** Florida
***Cephalosphorium lecanii* Zimm (*Verticillium lecanii*)**	**Entomophatogenic fungus**		**CA**	**Rivero-Aragon & Grillo-Ravelo (2000)** **Xie et al. (1988)** cited in **Hall (2008)** Cuba, China
***Cheilomenes sexmaculata* Fab. (Coleoptera: Coccinellidae)**	**Ocassional predator**		**CA**	**Hall (2008)** India
***Chilocorus nigrita* (Fab.) (Coleoptera: Coccinellidae)**	**Ocassional predator**		**CA**	**Hall (2008)** India
***Chilocorus cacti (L.)* (Coleoptera: Coccinellidae)**	**Ocassional predator**			**Miranda et al. (2008)**
Chrysopa boninensis* Okamoto *(Neuroptera: Chrysopidae)	**Generalist predator**		**CA**	**Yang et al. (2006)** cited in **Hall (2008)** China
Cycloneda sanguinea (L.)	**Predator**	**Nymphs**	**CA**	**Michaud & Olsen (2004)** **Miranda et al. (2008)** Florida

(Coleoptera: Coccinellidae)				
***Cladosporium* sp. nr. *oxysporum* Berk. & M.A. Curtis**	**Entomophatogenic fungus**	**Nymphs**	**CA**	**Aubert (1987** cited in **Halbert & Manjunath (2004)** **Hall (2008)** Florida
***Coccinella* rependa Thunberg (Coleoptera: Coccinellidae)**	**Generalist predator**		**CA**	**Hall (2008)** India
***Coccinella septempunctata* L (Coleoptera: Coccinellidae)**	**Generalist predator**		**CA**	**Hall (2008)** India
***Coelophora inaequalis* (F.) (Coleoptera: Coccinellidae)**	**Predator**	**Young nymphs**	**CA**	**Étienne et al. (2001)** Guadeloupe islands
***Curinus coeruleus* Mulsant** (larvae and adult females)	**Predator**	**Nymphs**	**CA**	**Michau & Olsen (2004)** **Hall (2008)** Florida
***Cycloneda sanguinea* L.** (adults and larvae)	**Predator**	**Nymphs**	**CA**	**Michaud (1999, 2002a)** cited in **Michaud & Olsen (2004)** **Hall (2008)** Florida
***Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae)**	**Parasite: Endoparasitoid** (well-known parasitoid)	**Nymphs**	**CA**	**Étienne, et al. (2001)** **Hall, E.G. (2008)** India, Philipine, Viet-nam, China, Guadeloupe islands, Réunion Island, Taiwan, Florida
***Exochomus childreni* Mulsan** (larvae)	**Predator**	**Nymphs**	**CA**	**Michaud, J.P. & Olsen L.E. (2004)** **Hall (2008)** Florida
***Harmonia axyridis* Pallas** (larvae and adult females)	**Predator**	**Nymphs**	**CA**	**Michaud & Olsen (2004)** **Halbert & Manjunath (2004)** **Hall (2008)** Florida
***Hibana velox* (Becker)**	**Predator**		**CA**	**Michaud (2004)** cited in **Hall (2008)** Florida
***Hirsutella citriformis* Speare**	**Entomophatogenic fungus**	**Nymphs and adults**	**CA**	**Étienne et al. (2001)** **Rivero-Aragon & Grillo-Ravelo (2000)** **Subandiyah et al. (2000)** **Étienne et al. (2001)** cited in **Hall (2008)** México, Indonesia, Cuba, Guadeloupe islands
Isaria fumosorosea* Wize *(= Paecilomyces fumosoroseus)	**Entomophatogenic fungus**	**Nymphs**	**CA**	**Subandiyah et al. (2000)** cited in **Hall, E.G. (2008)** Indonesia

				México, Cuba
Lecanicillium lecanii R. Zare & W Gams	**Entomophatogenic fungus**	**Nymphs**	**CA**	**Subandiyah et al., (2000)** **Meyer et al. (2008)** **Hunter et al. (2011)** Indonesia México, Cuba
Olla v-nigrum **Mulsant** (larvae and adult females)	**Predator**	**Nymphs**	**CA**	**Michaud & Olsen (2004)** **Hall (2008)** Florida
Psyllaephagus diaphorinae **Lin & Tao**	**Parasite**	**Nymphs**	**CA**	**(Viraktamath & Bhumannavar, 2002)** cited in **Halbert & Manjunath (2004)** Philippines
Saprinus chalcites Illiger	**Predator**	**Nymphs**	**CA**	**(Al-Ghamdi 2000)** cited in **Halbert & Manjunath (2004)** Saudi Arabia
Species of *Scymnus* (Coccinellidae)	**Predator**		**CA**	**(Gravena et al. 1996)** cited in **Halbert & Manjunath (2004)** Brazil
Allograpta spp. (Ex. Allograpta obliqua **Say*)***	**Predator**	**Nymphs**	**CA**	**(Aubert, 1987; (Michaud 2002)** cited in **Hall, E.G. (2008)** Réunion, Nepal, Florida
Syrphophagus taiwanus **Hayat & Lin**	**Parasite: Hyperparasite**	**Nymphs**	**CA**	**(Viraktamath & Bhumannavar, 2002)** cited in **Halbert & Manjunath (2004)** Taiwan
Tamarixia radiata **(Hymenoptera: Eulophidae)**	**Parasite: idiobiont, ectoparasitoid**	**Nymphs**	**BCD**	**Étienne, et al. (2001).** **Hall, E.G. (2008)** India, Guadeloupe Islands Mauritius, Réunion, Island, The United States (Florida and Texas), Taiwan, Brazil, Viet-nam, Puerto rico, Arabian Peninsula.
Ocyptamus sp. **(Diptera: Syrphidae)**	**Predator**	**Nymphs**	**CA**	**Miranda et al. (2008)**
Zelus longipes **L.**	**Ocassional predator**		**CA**	**(Hall et al. 2008)** cited in **Hall, E.G. (2008)** Florida

***Type of NE:** Predator (Pred), Parasite (Par), Entomophatogenic (Entomo)
***Current state of application of the natural enemy (CSA):** Research (Re), Cited application (CA), Biological control demonstrated
with results in the field (BCD)

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3. Installation of insect hotels and nest boxes

Project title: Development of sustainable control strategies for citric under threat of climate change & preventing entry of HLB in EU

1.2. Installation of insect hotels and nest boxes

Trioza erytreae	*Diaphorina citri*

1.2

- Presence of parasitoid and predatory insects
- Design Considerations
- Build Insect Hotels
- Create nesting blocks
- Insect Hotel Location
- Enhancing Habitat
- Maintaining Insect Hotels

1.2. Installation of insect hotels and nest boxes.

The insect hotel (nest box, box, house), also known as a bug hotel or insect house, is a manmade structure created to provide shelter for insects. Insect hotels can offer places for beneficial insects and pollinators to survive winter's chill and to nest in spring and summer.

Amongst the most common guests in these structures we can find solitary bees, lacewings, bumblebees or ladybirds. There are variety of shapes and sizes depending on the specific purpose or specific insect it is catered to. Most consist of different sections that provide insects with nesting facilities. In general, insect hotels favor the ecosystem, pollination and biodiversity, creating an environment that favors the balance between a large number of botanical species and a large number of animal species

The pollinator activity is mainly carried out by insects belonging to the orders Coleoptera (beetles), Lepidoptera (diurnal and nocturnal butterflies), Diptera (flies) and especially Hymenoptera, order in which besides bees and bumblebees, wasps and ants are grouped.

Figure 1. Images corresponding to the pollinator activity in citrus fruits.

In the last decades it is being observed a strong regression of bees and other pollinating insects. This not only represents a regrettable loss of biodiversity but also a serious threat to humans since the vast majority of crops depend on their pollinating function. This regression can be mostly attributed to the abuse of pesticides, such as neonicotinoids, as well as other toxic chemical agents.

Presence of predatory insects and parasitoids

The hotels aim to attract predatory insects that contribute to the control of unwanted pests in crop areas and green areas. There are many examples of this type of biological control, such as earwigs, whose presence in fruit trees and near them is very positive because they feed on aphids (a terracotta pot hung upside down, full of packages of straw or wool wooden offers an ideal housing for earwigs). Ladybugs, which are biological pest controllers par excellence, are easy to attract when many twigs are placed inside a wooden box (open on one side to provide many small cavities). Some species of ladybugs prefer to hibernate in larger groups, so insect hotels could be an attraction for these predators as a place of hibernation.

What types of predatory insects and parasitoids could we attract?

- ✓ Ladybirds overwinter as adults under branches and in log cavities. However, upon release they rarely stay in the area you intended. By offering overwintering habitats and alternative food supplies we may entice them to stay in the desired green or crop area.
- ✓ Parasitic wasps can burrow into trees, so including wood blocks with holes may lure them to your insect hotel. Although most adults feed on nectar or honeydew, they lay their eggs in the bodies of pests found in the green area or crop.
- ✓ Spiders overwinter as adults; they may stay active even at cold temperatures but seek out shelter to stay warm. They usually hibernate as immatures states under loose bark or as egg cases.
- ✓ Hoverflies overwinter as pupae in leaf debris, pinecones, and straw. These species are pollinators as adults, but their larvae are ferocious hunters of aphids. It necessary good sources of nectar and pollen for the adults to be attracted.
- ✓ The hoverflies spend the winter like pupae in remains of leaves, pineapples and straw. These species are pollinators in an adult state, but their larvae are fierce aphid hunters. If there are adequate sources of nectar and pollen, adults can be attracted to our hotel.
- ✓ Bees overwinter in nesting cavities (hollow cavities) and spend at least 10 months going from egg to adult. These nesting cavities can be made of bamboo sticks, hollow-stemmed plants like sunflowers, or holes drilled in wood. Inside the holes, the eggs are hatching, and the little larvae are consuming pollen. In some insect hotels these nesting cavities are placed in old drainage tiles to keep them dry.
- ✓ Green lacewings prefer to pupate in rolled-up corrugated paper.

Design Considerations

The most well-known artificial dwellings are simple wooden blocks drilled with holes. Hollow stems also work well; leave a node at one end to provide a “back wall”. Stems of fennel, elderberry, bamboo, brambles, teasel, and many members of the buckwheat family (Polygonaceae) are all suitable. Stems of approximately the same diameter (about 5-6 inches long or 12.7-15.24 cm) can be tied in bundles or packed into cans, pipes, or other objects and used either singly or incorporated into larger structures. Wet clay or drilled dry clay blocks can be added to insect hotels as shelter for a variety of hymenoptera.

Other materials suitable for experimentation includes lichen, straw, bark, twigs, leaves, pinecones and other organic materials. Lacewings and other insects can use rolled-up corrugated cardboard as hibernation shelter. The use of dry materials is especially important in damp or foggy areas.

Building and location of Insect Hotels

A hotel can be fashioned from a simple box or can or designed as an elaborate structure requiring the services of a professional builder. Regardless of size, the hotel must be firmly attached to its base, and level. The shape and size of the "insect hotel", its orientation and the choice of its location are very important. It is advisable to direct the shelter towards a direction where it is protected from the wind, near a cultivated space, a mountain, an orchard or wild flowers. It must be at least 30 cm from the ground and offer shelter in case of bad weather. One way of protecting the hotel from a storm direction would be by placing it under an eave or providing a sheltering roof.

Position hotels to receive morning sunlight; east or southeast positions are preferred. West-facing hotels may get too hot for most insects. If more than one hotel is being installed, experiment with locations and observe the different species each hotel attracts since resident species may have different temperature and relative humidity requirements.

It is important to stress that without objective information insect hotels are often badly designed and they offer unsuitable home to the target insects. The warning sign of such designs is the unnecessary use of pinecones, glued snail shells, wood shavings and clear plastic tubes. Too many off-shelf insect hotels or build-your-own websites do not come with clear guide on maintenance, which is very important in ensuring the survival of the insects we intend to host (Teh-Weisenburger, 2017).

Insect hotel locations not far from a cultivated plot, flower bed, orchard or wild flowers will maximize its function: it will be the hotel restaurant!

Figure 2. Comparison between non-ideal (left) and ideal insect hotels (right) (Image from David Werner). Source of image of David Werner: https://www.naturgartenfreude.de/wildbienen/nisthilfen/schautafeln

The composition of rooms depending on the type of insect that is meant to be housed can be the following (Nadreau, s.f.):

- For lacewings: a red box stuffed with straw with some openings.
- For bumblebees: a box with a hole of 10 mm in diameter.
- For certain solitary bees and wasps: a braided cane mat.
- For solitary bees: bricks with holes filled with a mixture of clay and straw.
- For diptera (syrphids): some dry wood with holes like bamboo stalks.
- For earwigs: a pot turned and full of wood fibers or hay.

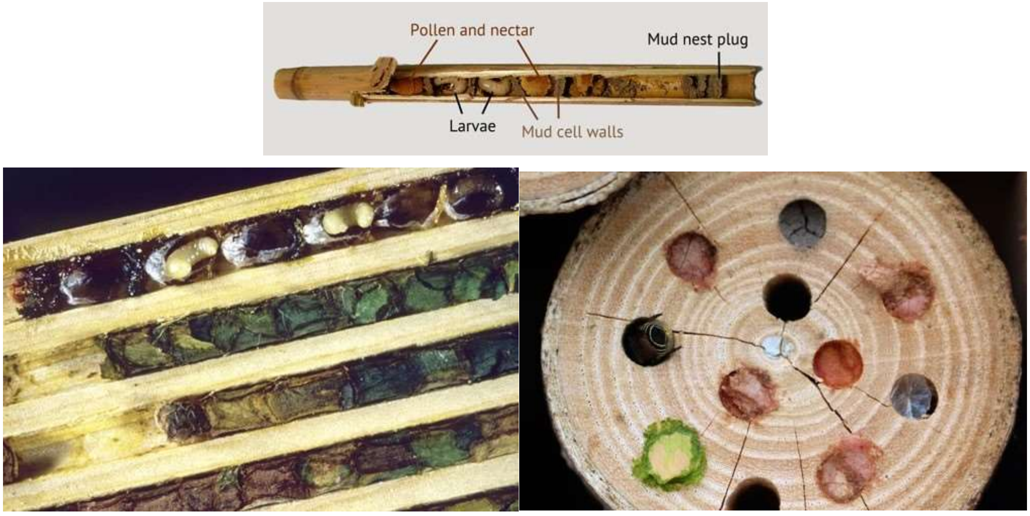

Figure 3. A nest cavity cross-section, showing bee larvae and nest materials and capped nest entrances. Source of images: NebGuide University of Nebraska–Lincoln Extension, Institute of Agriculture and Natural Resources (2015)

Also, the trunks are very popular shelters for many pollinators such as bees or solitary wasps, and the gaps between wooden boards can attract the ladybugs who come to spend the winter there.

Create nesting blocks

To encourage nests of different bee species, blocks of wood should be up to 6 inches or 15,24 cm in length, with tunnels that can be various hole diameters, for example: 1/2 – 3/16 inches or 1.27 - 0.47 cm, drilled into them (Figure 4). Hole diameter determines the depth of a tunnel, which should not breach the back of the wood block. The larger the diameter, the deeper the tunnel needs to be drilled. In addition to wood blocks, logs, or posts, bamboo or pithy reed grasses, ½ inches (1,27 cm) in diameter and under, may be used as nesting structures. Holes of varying diameters provided by these tubular plants will help to attract different species of solitary bees (Bauer & Lynch, et al., 2015).

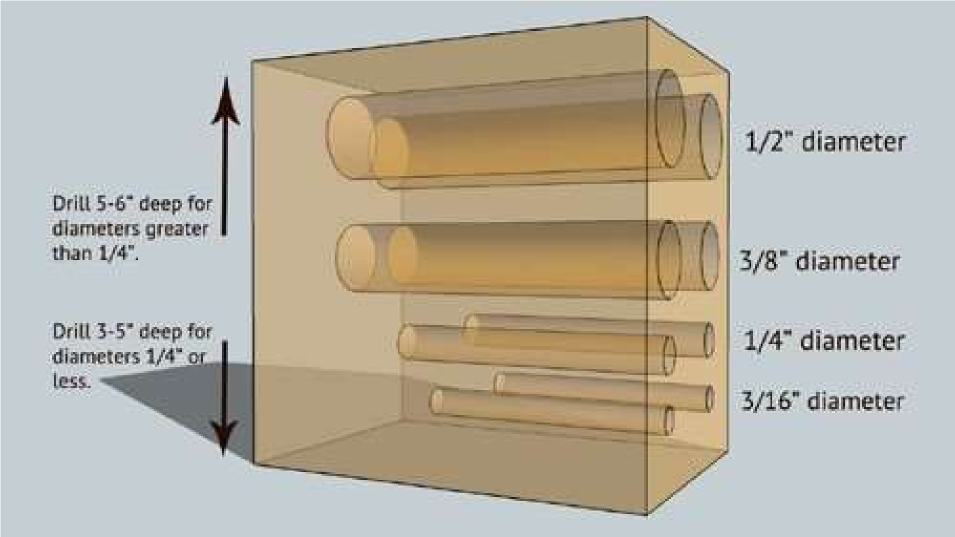

Figure 4. Hole diameter affects the type of bee or insect that will be at- tracted to the nesting block. Depth depends on the diameter of the hole, with larger diameters requiring deeper drilling. Source of images: NebGuide University of Nebraska–Lincoln Extension, Institute of Agriculture and Natural Resources (2015).

The nesting blocks and tubular plant materials should be placed and packed tightly in the bee hotel frame. Do not use glue to secure nesting materials to the frame, as these materials need to be replaced yearly.

It is also important to provide the nest with some colour, especially blue or violet to further attract the attention of potential and future tenants: for example, the bees, to which the blue and violet, especially draw their attention.

Enhancing Habitat

In addition to nesting sites insect hotels, other habitat resources should be included. A source of water, flowering plants that can feed insects, overwintering sites, and shelter from the wind are important in attracting a diversity of beneficial insects. Areas of wet clay provide a resource of mud nest-building predatory wasps and for “mud-puddling” butterflies. Brush and leaf piles, and bunch grasses are all attractive to overwintering insects; trees with loose or fissured bark provide winter shelter for butterflies and other insects.

Figure 5. In addition to nesting sites insect hotels, other habitat resources should be included important in attracting a diversity of beneficial insects, like example: flowering plants that can feed insects, a source of water, overwintering sites, and shelter from the wind, etc. Source of images: Experimental area of ecological crop at ICIA (Valle de Guerra, Tenerife).

Beneficial insects require flowering plants; besides bees and other familiar pollinators, many predatory insects use pollen and nectar as food in their adult stage, feeding live prey only to their larvae. Sequential flowering throughout the season in the area will ensure alternative food resources for natural enemies (hedges, crop surroundings, association of plants for the maintenance of native auxiliary fauna, etc). Must choose flowers with accessible nectar and pollen, such as plants in the daisy, carrot, mint, and mustard families. Bunch grasses are an important source of early season pollen for many insects. Other good choices are members of the rose and buckthorn families and native wildflowers; include a high proportion of locally native plants for best results.

"Layers" of forbs, grasses, shrubs, and trees create microhabitats, even in a designed landscape, and promote insect biodiversity. Layers also provide homes for alternate prey-food sources for predatory insects that will keep them in the area once they have eaten all their favored prey. Many insects are associated with particular species of host plants that can be incorporated. Let natural leaf mulch build up under native trees and shrubs and wait to cut back perennial plants.

Maintaining Insect Hotels

Insect hotels require maintenance for success, as some diseases and pests, such as mites, can build up in the facility after a few years. Some hotel builders advocate completely changing the filler materials every two years, or sanitizing drilled wooden blocks. Many pest and disease problems can be reduced by making small, disposable homes, but place new ones in the same location for insects that use the same site year after year.

Ants can be controlled with borax preparations applied at the base of an insect hotel. If woodpeckers become a problem (some authors suggest that an "insect hotel" could be easily become a "woodpecker restaurant"), consider installing a chicken wire barrier around the hotel.

Large insect hotels (aptly called insect condominiums) using wooden pallets are becoming very popular as individual or community gardening projects, sometimes to include non-insects such as frogs, toads and hedgehogs. In contrast, natural insect habitats occur as small separate nests, and large insect hotels pose risk of disease and parasitism to the insects inhabiting in high density inside. In fact, Rosita Moenen (2012) observed that increasing number of badly-designed artificial nesting sites contributed to higher loss of (solitary) bees by parasitism (Teh-Weisenburger, 2019).

Insect hotels (especially large ones) are very susceptible to parasitism (Moenen, 2012; Macivor & Packer, 2015). When not managed, the parasites will end up spreading to the rest of the insect hotels and will continue on for following seasons. In similar note, mould brings diseases to insects. It grows when moisture condenses and gets trapped in plastic materials used in insect hotels as tubes and blocks (Carlton, 2015). Lack of good roof/shelter on insect hotels, risking constant exposure to rain also contributes to mould growth (Teh-Weisenburger, 2019).

The right approach to insect hotels is the following (Teh-Weisenburger, 2019):

- Be realistic – small is better: Assess your area where you plan to set up your insect hotel or refuge. Think small and have multiple units housing one species rather than a single large one that attempts to host an entire zoo, requiring potentially conflicting environments.
- Choose responsible design: There are a number of good guides online written by entomologists for non-bee hotels suitable for lady bugs, lace wings and non-migrating butterflies, Melanie von Orlow (2019) has written a book with detailed manuals, available in Dutch and German.
- Build it right: with materials like natural, untreated wood and without chemicals such as varnish, paint and wood protectant that will repel insects. The construction material will be treated with natural waterproofing substances such as virgin wax or oils, avoiding those that generate rejection of insects. To promote sustainability, consider using recycled or natural materials. If tubes are drilled into blocks, tubes should be smooth without splinters. Good insect hotels should be built sturdy with solid back and roof/shelter to protect from rain. The shelter must be well insulated and protected from moisture.
- Install it well: For example, bee hotels must be positioned in full sun, facing south east or south, at least a metre off the ground, with no vegetation in front of it obscuring the

entrances to the tunnels. It must also be fixed securely to prevent shaking and swaying from wind.

- Maintain and clean: This is the most overlooked part of having insect hotel. Taking care of insect hotel is just as important as building one. For example, bee hotels should be inspected at the end of summer to remove and clean dead cells. The clean will prevent mould and mites that would multiply on the dead bees or larvae. Some experts recommend bringing occupied insect hotel into cool dry area during winter to protect the overwintering inhabitants from wind and rain. Without timely maintenance and clean-up, a once-occupied insect hotel may not attract a new batch next season.
- Replace when it is time: Insect hotels can degrade naturally after two or more years because the material used is untreated. Change the nesting blocks or parts every two years to avoid build-up of mould, mites and parasites overtime.






Figure 6. Several examples of insect hotel constructions, with different sizes and shapes, and inserted in different environments. Source of images:
NebGuide University of Nebraska–Lincoln Extension, Institute of Agriculture and Natural Resources (2015)
https://lighthouse.mq.edu.au/_resources/images/articles/march-2019/insect-hotel-inside700x400.jpg
https://s3-us-west-2.amazonaws.com/hobbyfarms.com/wp-content/uploads/08095306/insect-hotel7_Moira-Dunworth.jpg
https://www.google.com/url?sa=i&source=images&cd=&ved=2ahUKEwjKmumv6PDlAhULLBoKHdFcDI4QjRx6BAgBEAQ&url=https%3A%2F%2Fwww.plant-theatre.co.uk%2Fbee-hotel-and-flower-seeds.html&psig=AOvVaw0mSXpWX9zO9mKXJ45HAVHU&ust=1574063034647717
https://www.google.com/url?sa=i&source=images&cd=&ved=2ahUKEwi_mMOv6fDlAhVB-YUKHWFDAfgQjRx6BAgBEAQ&url=https%3A%2F%2Fwww.brabantslandschap.nl%2Factueel%2Fnieuws%2Fnederland-zoemt%2F%3Fregion%3Dall%26area%3Dall&psig=AOvVaw0mSXpWX9zO9mKXJ45HAVHU&ust=1574063034647717

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- **Bauer E.C., Lynch, L.L., et al. (2015).** Creating a solitary Bee Hotel. NebGuide University of Nebraska–Lincoln Extension, Institute of Agriculture and Natural Resources (2015). Available in: http://extensionpublications.unl.edu/assets/pdf/g2256.pdf
- **Lavoipierre Frederique.** Web Extra: A Resource Guide to insect hotels. Available in: https://www.pacifichorticulture.org/articles/web-extra-a-resource-guide-toinsect-hotels/
- **Macivor, J. S., & Packer, L. (2015).** Bee Hotels' as Tools for Native Pollinator Conservation: A Premature Verdict? Plos One, 10(3). doi: 10.1371/journal.pone.0122126.
- **Moenen, R. (2012).** De broed parasiet *Cacoxenus indagator* (Drosophilidae) en de parasitoïden *Melittobia acasta* (Eulophidae) en *Coelopencyrtus sp.* (Encyrtidae) bij solitaire bijen in kunstmatige nestgelegenheid. *Entomologische Berichten*, 1-2, 63-70. Retrieved September 8, 2017, from https://www.nev.nl/pages/publicaties/eb/nummers/2012/72-1-2/63-70.pdf.

- **Nadreau, J. (s.f.).** Hotel de Insectos Urbanos. Documento producido por Julien Nadreau, Hortelano de "Jardins des Aures" y miembro de RJSM durante el proyecto EU'GO. Available in: https://www.eugolearning.org/sites/default/files/files/documents/insect_house_es.pdf
- **Teh-Weisenburger, Jo-Lynn (2019).** Insect Hotels: A Refuge or a Fad? Available in:https://entomologistlounge.wordpress.com/2017/09/18/insect-hotels-arefuge-or-a-fad/
- **Von Orlow, Melanie (2019)**. Bee Hotel: All you need to know in one concise manual. HAYNES PUBLISHING GROUP. J H Haynes & Co Ltd. 94p.

Images sources

- Bee hotel comparison by David Werner. Available in: https://www.naturgartenfreude.de/wildbienen/nisthilfen/schautafeln
- NebGuide University of Nebraska–Lincoln Extension, Institute of Agriculture and Natural Resources (2015). Available in: http://extensionpublications.unl.edu/assets/pdf/g2256.pdf

Links to interesting information on the subject: Hotels for insects

- POLLINATOR PARTNERSHIP. http://www.pollinator.org/gardens.htm

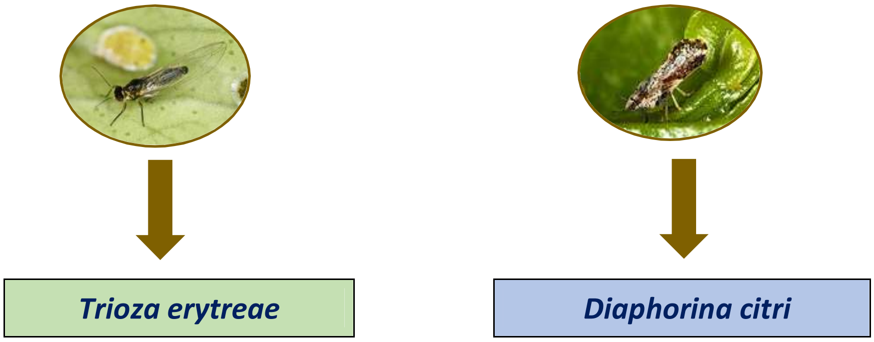
- UNIVERSITY OF NEBRASKA – LINCOLN EXTENSION, INSTITUTE OF AGRICULTURE AND NATURAL RESOURCES. http://extensionpublications.unl.edu/assets/pdf/g2256.pdf
- CONSTRUCTOR DE HOTELES PARA POLINIZADORES. http://www.projectebrot.com/obras/insect-hotels/7/S/
- CAJAMAR e IFAPA. App PlantEn para la biodiversidad funcional en los cultivos. https://apps.apple.com/es/app/planten/id1381507079 https://play.google.com/store/apps/details?id=com.upwarestudios.upws011
- PERMACULTURE RESEARCH INSTITUTE. Building an Insect Hotel. https://permaculturenews.org/2013/10/08/building-insect-hotel/

Links to videos related to this topic: Hotels for insects

- https://youtu.be/2OKKiBbbQ3s
- https://youtu.be/_0EXEstcA9Q

4. Alternative host plants: Identification of refuge plants for potential natural enemies of the main citrus pests

1.3. Alternative host plants: Identification of refuge plants for potential natural enemies of the main citrus pests.



1.3. Alternative host plants: Identification of refuge plants for potential natural enemies of the main citrus pests.

The natural biological control of pests by the community of natural enemies (native auxiliary fauna) that are present in agroecosystems, constitutes one of the ecosystem services of greater economic value for agriculture worldwide. Currently, it is estimated that natural enemies of plague insects are responsible for between 50% and 90% of natural biological control in crop fields (Pimentel, 2005; Rodríguez Navarro & González Fernández, 2014).

Conserving and increasing the populations of these native insects, through habitat management, is the primary objective of biological conservation control. Beneficial insects improve their growth, development, survival and/or fertility by exploiting the plant resources that plants offer them. In plants, natural enemies not only find refuge from adverse weather conditions and/or from predators, they also find preys and alternative hosts, especially when they are scarce in the fields. In addition, most of the natural enemies of the plagues are not strict predators, but rather have a high degree of omnivory. Thus, at some point in their biological cycle, either as a larva, as an adult, or in both cases, they depend on the food resources that plants offer them in the form of nectar (floral or extrafloral), pollen, seeds, juices, or molasses produced by phytophagous insects.

Within the selection criteria used to identify which plants can be potentially useful to attract and maintain the key natural enemies of agricultural pests are the following (Rodríguez Navarro & González Fernández, 2014):

- **Use native plants that are commercially available in nurseries.** The viability of a hedge, that its survival, repopulation is feasible and that its establishment is carried out in the shortest possible time, is a key factor when designing a plant barrier. The use of native plants ensures this viability, since these are perfectly adapted to the edaphoclimatic conditions of the area in question, are less invasive, and have an easier handling than the native plants. In addition, it is known that native vegetation ensures better crop protection since it is less susceptible to attack by pests and / or diseases than cultivated or ornamental plants (Bianchi et al., 2013).

- **Use plants that are not reservoirs of viral diseases:** A viable option is to use native shrubby plants that do not play this role.
- **Use plants that offer alternative food resources for example in the form of pollen and / or nectar.** In general, the literature points out as families that produce pollen to the *Cruciferae, Cistaceae* and *Compositaceae,* and as producers of nectar, to the families *Lamiaceae, Borraginaceae*, *Scrophularicaeae, Ericaeae, Apiaciae (umbelliferae)* and some *Fabaceae*.

- **Use plants that offer shelter and/or carry extrafloral nectaries:** There are certain aspects of the morphology of a plant, such as extrafloral nectaries, or the presence of trichomes on the leaves, which can greatly influence phytophagous insects, natural enemies that they attract and their interactions. Extrafloral nectar is an important source of food, independent of flowering, for natural enemies with importance in the biological control of pests such as: crisopas, phytoseids, parasitoids or predatory flies (Koptur, 2013). On the other hand, it has been shown that the presence of trichomes in the leaves favors, for example, the installation of populations of phytoseid mites; as a refuge to avoid adverse abiotic conditions or to hide from predators, they also facilitate the increase of pollen capture and its subsequent use as a food source (Loughner et al., 2010). In general, plants belonging to the genus *Prunus* and the families *Fabaceae* and *Euphorbiaceae* usually have extrafloral nectaries.
- **Use shrubby plants:** Compared to other habitat types, shrub hedges are the ones that offer the greatest amount of resources to natural enemies of pests, and there is evidence that they improve biological control in adjacent crop fields (Holland, 2012).

Citrus fruits are evergreen trees, and can host a great diversity of auxiliary entomofauna (Soler et al., 2002, Puig-Ochoa, 2018). Therefore, knowledge in this regard and the development of pest management techniques by biological control has been especially important in citrus farming. Conventional citrus farming, given the type of active materials used and its mode of application, can seriously affect the natural enemies that allow pest self-regulation (Miret and García Marí, 2001). The methods of Integrated Pest Management and ecological management result in a management based on the minimum application of pesticides, according to threshold criteria, monitoring, sampling, and with extensive support in biological control methods (Garcia-Marí, 2009; Ramírez Ferrer, 2019).

Many of the main citrus pests (Table 1) have adequate natural biological control, although historically they have resorted to introductions of natural enemies; Two relevant examples are the establishment of insects of the Generalitat Valenciana for the release of predatory coccinellids as a flood control measure or the introduction of natural enemies such as *Citrostychus phillocnistoides* Narayan against the miner *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillaridae), among others (Vercher et al., 2000; Van der Blom, 2002; Jacas et al., 2006) (Ramírez Ferrer, 2019).

Table 1. Main pests reported in citrus fruits (Soler J.M, 2019 (Bayer)). Natural enemies, calendar and sampling methods.

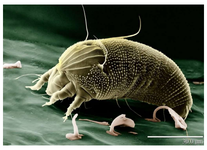
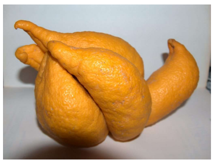
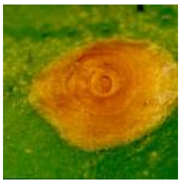
	PLAGUE Common name (Scientific name)	Sampling calendar (Maximum Population ■) Sampling Methods and DamageThresholds	Natural enemies
Mites	Citrus Red Mite (*Panonychus citri*) 	Jan, Feb, March, April, May, June, July, August ■, Sept ■, Octob, Nov, Dec Sampling every two weeks. Two fully formed leaves of the last sprout per tree should be sampled, determine number of leaves occupied by red mite. In addition, sample a mature leaf from the inside and determine the number of leaves occupied by phytoseids. Threshold: Treat when nº leaves with phytoseids < 30% and nº leaves with red mite>20% between august and october.	 **Controlled naturally by the fitoseido *Euseius stipulatus*** *(predator)*
	Spider mites Genus *Eutetranychus* *(E. orientalis* y *E. banksi)*  Female of *E. orientalis*	Jan, Feb, March, April, May, June, July ■, August ■, Sept ■, Octob, Nov, Dec To detect its presence in the field, it should be taken into account that, at the beginning of the colonization, the population is more abundant in the South-Southeast orientation of the tree and in the outer and upper part of the tree, it also has a preference for the central nerve of the beam of the leaves.	**Phytoseiid predators: *Euseius stipulatus, Neoseiulus californicus, Phytoseiulus persimilis and Typhlodromus* sp.**   **(a)** **(b)** **(a)** ***N. californicus*** **(b)** ***P. persimilis*** predating
	Red spider mite or two-spotted spider mite *(Tetranychus urticae)* 	Jan, Feb, March, April, May, June, July ■, Augus ■, Sept ■, Octob, Nov, Dec Sampling with weekly or biweekly frequencies. Sampling is done by depositing two 56 cm Ø rings on the tree canopy and counting the number of "occupied rings", those that contain two or more symptomatic leaves (yellow spots). Four symptomatic leaves are also sampled and the number of leaves occupied by red spider is determined.	No effective natural enemies are known, although phytoseid mites are often seen among their colonies (***Neoseiulus californicus y Phytoseiulus persimilis)*** and larvae and adults of coleoptera (coccinellids) ***Stethorus punctillum.***  ***Stethorus punctillum***
	Citrus bud mite *(Aceria sheldoni)*  	Jan, Feb, March, April ■, May ■, June, July, August, Sept, Octob ■, Nov, Dec For sampling, make two annual observations, in autumn and spring, before the new shoots reach 5 cm. Observe in four trees four complete branches (or 30 cm from the end) per tree. The branches will be of the last budding fully developed. In each branch observe: 1) visually in the field, presence of deformations 2) in the laboratory, at the binocular, presence of mites under the bracts of the buds. The intervention time will be when 25% of the branches show deformations or when the presence of mites is observed in 50% of the buds. If you decide to intervene, perform the application when most of the tree buds are between 4 and 6 cm.	Some species that attack the red spider, such as ***Amblyseius californicus*** o ***Stethorus puncttillum***. However, the biological control of this pest is currently quite limited, having to resort to chemical treatments.

Table 1 – Continuation: Main pests reported in citrus

<table>
<tr><td rowspan="6">Hemiptera: Diaspididae</td><td rowspan="3">California Red scale (Aonidiella aurantii)

Adult female

Adult male</td><td>Jan</td><td>Feb</td><td>March</td><td>April</td><td>May</td><td>June</td><td>July</td><td>August</td><td>Sept</td><td>Octob</td><td>Nov</td><td>Dec</td><td rowspan="3">Predators: Rhyzobius lophanthae and Chilocorus bipustulatus, Scymnus subvillous, Scymnus interruptus, Pilophorus gallicus

Adult and y larva of R. lophanthae
In countries where the California red louse is controlled naturally, it is by parasites of the genus Aphytis. In addition, in those places where the control does not work naturally, the massive release of the parasitoid A. melinus is presented as a perfect tool for its control.

Adult and larva of Chilocorus bipustulatus.</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td colspan="12">Sampling during collection, observe 200 random fruits and determine a % of fruits attacked.
- In mid-august, carry out periodic sampling to determine the maximum of sensitive forms (young nymphs). For this, the percentage of fruit infestation must be determined (200 random fruits in 50 trees, 4 fruits / tree).
Thresholds:
If 2% or more of affected fruit is observed in the previous campaign it is recommended to treat to the maximum to sensitive forms in the first generation.
- In pending harvest, if 2% or more of affected fruit is observed in the second generation, treat the sensitive forms (young nymphs) with oil.</td></tr>
<tr><td rowspan="3">Oleander scale (Aspidiotus nerii) (lemon Tree)

Adults</td><td>Jan</td><td>Feb</td><td>March</td><td>April</td><td>May</td><td>June</td><td>July</td><td>August</td><td>Sept</td><td>Octob</td><td>Nov</td><td>Dec</td><td rowspan="3">Predators:
The coccinellids have been cited Chilocorus bipustulatus y Rhyzobius lophanthae.
Parasitoids: There are many species of hymenoptera that attack this pest, especially Aphytis melinus, A. chrysomphali, A. chilensis and Encarsia citrina.</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td colspan="12">For sampling follow the same recommendations given in the case of the California red scale.</td></tr>
<tr><td rowspan="3">Hemiptera: Mealybugs</td><td rowspan="3">Cotonet citrus mealybug (Planococcus citri)

Adults

Nymphs taken care of by ants</td><td>Jan</td><td>Feb</td><td>March</td><td>April</td><td>May</td><td>June</td><td>July</td><td>August</td><td>Sept</td><td>Octob</td><td>Nov</td><td>Dec</td><td rowspan="3">In those plots where the cotonet causes problems it is recommended to release predator Cryptolaemus montrouzieri in early spring. These releases can be complemented with the release of parasitoids Anagyrus pseudococci and Leptomastix dactylopii in june.

Adults and larvaes of Cryptolaemus montrouzieri predating cotonet</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td colspan="12">If populations of natural enemies are respected and the rise of ants to trees is avoided, intervention should not be necessary.
In the event that it is necessary to reduce populations rapidly and treatment is required, sampling should be done in august and september when the females are in the fruits:
- Estimate the presence of cotonet (N3 and females) in 130 fruits spread over more than 50 trees.

Threshold: Treat only if more than 20% of the fruits are infested with cotonet.</td></tr>
</table>

Table 1 – Continuation: Main pests reported in citrus

<table>
<tr><td rowspan="2">Hemipter</td><td rowspan="2">Chinese wax scale (Ceroplastes sinensis)</td><td>Jan</td><td>Feb</td><td>March</td><td>April</td><td>May</td><td>June</td><td>July</td><td>August</td><td>Sept</td><td>Octob</td><td>Nov</td><td>Dec</td><td rowspan="2">Polyphagous predators such as coccinellids and neuroptera can feed on nymphs.
Parasitoides:</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
</table>

Nymph

Young Nymph

It is important to sample those plots where the Chinese wax scale has caused damage in previous years. It must be done in late summer when the breeding females are in the branches.
Observe four shoots (branches and leaves) to determine the moment when the maximum number of individuals are detected in sensitive forms (nymphs).
Number of trees: 75 - 100.
Threshold: It will be treated only when 3 individuals / outbreak are exceeded and when 100% of eggs are reached.

Scutellista caeruela (Hymenoptera: Pteromalidae), whose larvae feed on the eggs of the chinese wax scale, and the ***Microterys nietneri (Hymenoptera: Encyrtidae)*** are common parasitoids in our citrus fruits.

Scutellista caeruela

Cottony cushion scale *(Icerya purchasi)*

Adults female

Nymphs and eggs

Jan	Feb	March	April	May	June	July	August	Sept	Octob	Nov	Dec

As there are no serious problems, no sampling methods or treatment thresholds have been developed.

It is controlled by a coccinellid ***Rodolia cardinalis,*** both larvae and adults feed in addition to adult mealybugs.

Larvaes and adults of ***R. cardinalis*** predating Cottony cushion scale.

Hemiptera: Aphids

Cotton aphid *(Aphis gossypii)*

winged adult

Jan	Feb	March	April	May	June	July	August	Sept	Octob	Nov	Dec

Sampling should be done weekly or biweekly, during spring sprouting in the plots. To determine the treatment threshold, two 0.25 m^2 (0.56 cm diameter) rings are placed on the surface of the tree canopy and the percentage of shoots attacked by aphids is determined.

Predators: Among **coccinellids** stand out of the genus ***Scymnus. Scymnus interruptus*** *and* ***S. subvillosus.***
It is also common to observe ***Chrysoperla carnea (neuroptera: Chrysopidae),*** and cecidomyiid fly ***Aphidoletes aphidimyza (Diptera: Cecidomyiidae)*** about aphid colonies.

Larvaes of *Scymnus* predating aphid colonies.

Larva of Crisopa predating aphids.

	 nymph colonies	Threshold: Treatments are recommended when 25% of outbreaks are attacked. They will be considered attacked shoots, shoots with the presence of aphids.	 Larva of **syrphid flies**  Larvaes of ***A. aphidimiza***

Table 1 - Continuation

Hemiptera: Aphids

Spirea aphid *(Aphis spiraecola)*


Adult aptera


Nymphs and adult

Jan	Feb	March	April	May	June	July	August	Sept	Octob	Nov	Dec
			■	■							

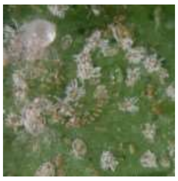
Sampling should be done weekly or biweekly, during spring sprouting in the plots. Procedure similar to sampling of ***Aphis gossypii.***

Similar to ***Aphis gossypii.***
Predators: Among coccinellids stand out of the genus *Scymnus*.
It is also common to observe ***Chrysoperla carnea (neuroptera: Chrysopidae),*** and cecidomyiid fly ***Aphidoletes aphidimyza (Diptera: Cecidomyiidae)*** about aphid colonies.

Hemiptera: whiteflies

Woolly whitefly *(Aleurothrixus floccosus)*


Adults


Nymphs

Jan	Feb	March	April	May	June	July	August	Sept	Octob	Nov	Dec
						■		■	■		

Sample in the summer (July) and fall (September-October) sprouting.
Woolly whitefly has a preference for young shoots, so it is recommended to observe the presence of fly individuals (adults, eggs and nymphs) in these shoots. The presence of parasitism should also be determined, especially by the parasitoid *Cales noacki*.
In general, it is recommended to sample 4 new shoots per tree.
Threshold: It is advisable to exercise some type of action against the pest if the level of infestation of the pest exceeds 20% of attacked shoots and the parasitism rate is less than 60%.

Various species of predators can exert a population reduction of the pest: coccinellids ***Clistotethus arcuatus*** and ***Cryptolaemus montrouzieri***, neuropterans ***Chrysoperla carnea*** and ***Conwentzia psociformis.***
Although they have low effectiveness for effective control because they are general predators.

It is an example of classic biological control, the parasitoid ***Cales noacki,*** that exerts total control of the pest since its introduction in our citrus fruits.


Pupa of ***Conwentzia psociformis***


Adult of ***C. psociformis***


Larva of ***C. psociformis***


Adult of ***Chrysoperla carnea***

Nesting whitefly

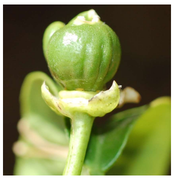
Jan	Feb	March	April	May	June	July	Augus	Sept	Octob	Nov	Dec

Polyphagous predators: family mites ***Phytoseiidae. Amblyseius* spp., *Euseius***

	(Paraleyrodes minei)  Adult  Larva													**spp., and *Typhlodromus* spp.,** They are active predators of eggs and nymphs of whiteflies. From the family of coccinellids ***Clitostethus arcuatus* (Rossi).**  Egg, larva, pupa and adult ***Clitostethus arcuatus***

Table 1 - Continuation

		Jan	Feb	March	April	May	June	July	August	Sept	Octob	Nov	Dec	
Hemiptera: Cicadellidae	**Smaller green leafhopper (Empoasca)**  													Several species of hymenoptera belonging to families stand out as parasitoids of Empoasca ***Dryinidae y Mymaridae*.** García Marí (2009) reported: ***Anagrus atomus***,L. (parasitoid), ***Coenosia attenuata***, S. (predador)  ***Coenosia attenuata***

		Jan	Feb	March	April	May	June	July	August	Sept	Octob	Nov	Dec	
Thysanoptera	**Kelly's citrus thrips (*Pezothrips kellyanus*)**  Adult  Nymph	Sampling should be done weekly from the total fall of the petals until the fruits have reached a diameter of 3.5-4 cm (6-8 weeks). Sampling healthy fruits and freshly set exteriors: Two fruits per tree. 25 trees on each side of the plot (100 trees). Threshold: The treatment will be carried out when 5% of fruits with the presence of nymphs of *P. kellyanus* are exceeded. This will determine the presence of nymphs in 100 healthy fruits (between 1 and 2 fruits per tree).  *Hypoaspis* sp.												The most important biological control agents of *P. kellyanus* are the predatory mites present in the soil. Among them highlights the mite Gaeolaelaps (*Hypoaspis)* sp. The natural enemies of *P. kellyanus* present in the treetops are mostly generalist predators (myrids, Chrysopids, coccinellids and other thrips) and don't seem to have a very important impact on the abundance of their populations.

		Jan	Feb	March	April	May	June	July	August	Sept	Octob	Nov	Dec	
Lepidoptera	**Citrus leafminer (*Phyllocnistis citrella*)**							■			■			**Predadors:** Many species of arthropods: ***Thrips* sp.** (Thysanoptera: Thripidae), ***Chrysoperla carnea*** (Neuroptera: Chrysopidae), ***Orius* sp.** (Hemiptera:

Larva

Pupa

adult

In seedlings and grafts, the observation of attacked receptive shoots (100 shoots in 50 trees, 2 shoots / tree) in summer and autumn shoots is recommended.
Threshold: In seedlings and grafts, treat when the presence of the leafminer is observed from the 2nd sprouting.
It is important not to perform chemical applications on trees in full production.

Parasitoids: *Citrostichus phyllocnistoides* is a main parasitoid of leafminer.

Adult

Egg on leafminer larva

Anthocoridae) and spiders (Araneae).
Generally prey leafminer in the absence of the species that they usually consume.

Nymph ***Orius* sp.**

Adult ***Orius* sp.**

Table 1 - Continuation

Hemiptera: Aphids

Spirea aphid *(Aphis spiraecola)*

Adult aptera

Nymphs and adult

Jan	Feb	March	April	May	June	July	August	Sept	Octob	Nov	Dec
			■	■							

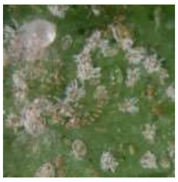
Sampling should be done weekly or biweekly, during spring sprouting in the plots. Procedure similar to sampling of ***Aphis gossypii.***

Similar to ***Aphis gossypii.***
Predators: Among coccinellids stand out of the genus *Scymnus*.
It is also common to observe ***Chrysoperla carnea (neuroptera: Chrysopidae),*** and cecidomyiid fly ***Aphidoletes aphidimyza (Diptera: Cecidomyiidae)*** about aphid colonies.

Hemiptera: whiteflies

Woolly whitefly *(Aleurothrixus floccosus)*

Adults



Nymphs

Jan	Feb	March	April	May	June	July	August	Sept	Octob	Nov	Dec
						■		■	■		

Sample in the summer (July) and fall (September-October) sprouting.
Woolly whitefly has a preference for young shoots, so it is recommended to observe the presence of fly individuals (adults, eggs and nymphs) in these shoots. The presence of parasitism should also be determined, especially by the parasitoid *Cales noacki*.
In general, it is recommended to sample 4 new shoots per tree.
Threshold: It is advisable to exercise some type of action against the pest if the level of infestation of the pest exceeds 20% of attacked shoots and the parasitism rate is less than 60%.

Various species of predators can exert a population reduction of the pest: coccinellids ***Clistotethus arcuatus*** and ***Cryptolaemus montrouzieri***, neuropterans ***Chrysoperla carnea*** and ***Conwentzia psociformis.***
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Pupa of ***Conwentzia psociformis***

Adult of ***C. psociformis***

<table>
<tr><td></td><td></td><td colspan="12"></td><td>Larva of C. psociformis</td><td>Adult of Chrysoperla carnea</td></tr>
<tr><td rowspan="3"></td><td rowspan="3">Nesting whitefly (Paraleyrodes minei)

Adult

Larva</td><td>Jan</td><td>Feb</td><td>March</td><td>April</td><td>May</td><td>June</td><td>July</td><td>Augus</td><td>Sept</td><td>Octob</td><td>Nov</td><td>Dec</td><td colspan="2" rowspan="3">Polyphagous predators: family mites Phytoseiidae. Amblyseius spp., Euseius spp., and Typhlodromus spp., They are active predators of eggs and nymphs of whiteflies. From the family of coccinellids Clitostethus arcuatus (Rossi).

Egg, larva, pupa and adult Clitostethus arcuatus</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td colspan="12"></td></tr>
</table>

Table 1 - Continuation

<table>
<tr><td rowspan="3">Hemiptera: Cicadellidae</td><td rowspan="3">Smaller green leafhopper (Empoasca)

</td><td>Jan</td><td>Feb</td><td>March</td><td>April</td><td>May</td><td>June</td><td>July</td><td>August</td><td>Sept</td><td>Octob</td><td>Nov</td><td>Dec</td><td rowspan="3">Several species of hymenoptera belonging to families stand out as parasitoids of Empoasca Dryinidae y Mymaridae. García Marí (2009) reported: Anagrus atomus,L. (parasitoid), Coenosia attenuata, S. (predador)

Coenosia attenuata</td></tr>
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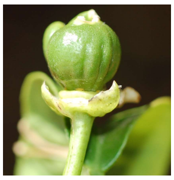
<tr><td colspan="12"></td></tr>
<tr><td rowspan="3">Thysanoptera</td><td rowspan="3">Kelly's citrus thrips (Pezothrips kellyanus)

Adult

Nymph</td><td>Jan</td><td>Feb</td><td>March</td><td>April</td><td>May</td><td>June</td><td>July</td><td>August</td><td>Sept</td><td>Octob</td><td>Nov</td><td>Dec</td><td rowspan="3">The most important biological control agents of P. kellyanus are the predatory mites present in the soil. Among them highlights the mite Gaeolaelaps (Hypoaspis) sp.

The natural enemies of P. kellyanus present in the treetops are mostly generalist predators (myrids, Chrysopids, coccinellids and other thrips) and don’t seem to have a very important impact on the abundance of their populations.</td></tr>
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Threshold: The treatment will be carried out when 5% of fruits with the presence of nymphs of P. kellyanus are exceeded. This will determine the presence of nymphs in 100 healthy fruits (between 1 and 2 fruits per tree).

Hypoaspis sp.</td></tr>
<tr><td rowspan="2">Lepidoptera</td><td rowspan="2">Citrus leafminer (Phyllocnistis citrella)</td><td>Jan</td><td>Feb</td><td>March</td><td>April</td><td>May</td><td>June</td><td>July</td><td>August</td><td>Sept</td><td>Octob</td><td>Nov</td><td>Dec</td><td rowspan="2">Predadors: Many species of arthropods: Thrips sp. (Thysanoptera: Thripidae), Chrysoperla carnea (Neuroptera: Chrysopidae), Orius sp. (Hemiptera:</td></tr>
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</table>


Larva


Pupa


adult

In seedlings and grafts, the observation of attacked receptive shoots (100 shoots in 50 trees, 2 shoots / tree) in summer and autumn shoots is recommended.
Threshold: In seedlings and grafts, treat when the presence of the leafminer is observed from the 2nd sprouting.
It is important not to perform chemical applications on trees in full production.

Parasitoids: *Citrostichus phyllocnistoides* is a main parasitoid of leafminer.


Adult


Egg on leafminer larva

Anthocoridae) and spiders (Araneae).
Generally prey leafminer in the absence of the species that they usually consume.


Nymph ***Orius* sp.**


Adult ***Orius* sp.**

Table 1 – Continuation: Emerging pests

Hemiptera

Cotonet de Les Valls (*Delottococcus aberiae*)


Fruit with population of *D. aberiae*


Adult male

Jan	Feb	March	April	May	June	July	August	Sept	Octob	Nov	Dec
			■	■	■						

- It is recommended to carry out field sampling between April and June, in more than 50 trees.
Threshold:
- Treat the fall of petals only if more than 12% of the fruits are infested with cotonet.

No effective predators or parasitoids have yet been found. The predatory coccinellid ***Cryptolaemus montrouzieri*** it can get to control the populations of ***D. aberiae.***


Larva of *Cryptolaemus montrouzieri* feeding of *D. aberiae*

Obscure mealybug Cotonet *(Pseudococcus viburni)*

Jan	Feb	March	April	May	June	July	August	Sept	Octob	Nov	Dec


Cryptolaemus montrouzieri


Larva of *Scymnus* spp.


Nephus sp.

Natural enemies include predators like ***Cryptolaemus montrouzieri*, Nephus sp. y *Scymnus* spp**. and nine species of parasitoid wasps.

In those plots where the cotonet causes problems it is recommended to release the predator ***Cryptolaemus montrouzieri*** in early spring. These releases can be complemented with the release of the parasitoids ***Anagyrus pseudococci*** and ***Leptomastix dactylopii*** in june.

<table>
<tr><td rowspan="3"></td><td rowspan="3">Cotonet
Long-tailed mealybug
(Pseudococcus longispinus)

</td><td>Jan</td><td>Feb</td><td>March</td><td>April</td><td>May</td><td>June</td><td>July</td><td>August</td><td>Sept</td><td>Octob</td><td>Nov</td><td>Dec</td><td rowspan="3">Predators: Beneficial insects with general feeding habits, such as Chrysopa, Cryptolaemus montrouzieri Muls., Sympherobius sp. (neuroptera).

In those plots where the cotonet causes problems it is recommended to release the predator Cryptolaemus montrouzieri in early spring. These releases can be complemented with the release of the parasitoids Anagyrus pseudococci and Leptomastix dactylopii in june.</td></tr>
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<tr><td colspan="12">
Sympherobius sp.

C. montrouzieri</td></tr>
<tr><td rowspan="3">Thysanoptera: Thripidae</td><td rowspan="3">Orchid thrips/
Citrus rust thrips or Anthurium thrip
(Chaetanaphothrips ochidii)

</td><td>Jan</td><td>Feb</td><td>March</td><td>April</td><td>May</td><td>June</td><td>July</td><td>August</td><td>Sept</td><td>Octob</td><td>Nov</td><td>Dec</td><td rowspan="3">The control of this citrus thrips has only been studied in Florida (Childers & Stansly, 2005).
Orchid thrips attacks all citrus species, especially Navel oranges, Valencia and red grapefruit varieties (Childers and Stansly, 2005). It is considered an important citrus pest in Florida.</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td colspan="12">The orchid thrips was identified long ago as a pest on Florida grapefruit by Thompson (1940). Re-occurrence of this problem likely is the result of pesticide substitutions away from ethion and other organophosphate insecticide uses applied during the postbloom and/or summer sprays. More specific acaricides such as Agri-mek + an HMO or dicofol, or an HMO applied alone have been substituted in the postbloom and/or summer sprays since the late 1980s to 1990. These spray programs were not effective in minimizing feeding damage to maturing fruit by the orchid thrips, D. trifasciatus, or the greenhouse thrips (Childers and Stansly, 2005).

This study validated the effectiveness of the alcohol wash method as a sampling method for estimating thrips numbers was validated. It was determined that a tentative action threshold of 3% of incipient damage is more realistic for grower usage, therefore, it could be a more practical treatment threshold level for monitoring than the 10% thrips incidence level by the visual method.</td></tr>
</table>

The incorporation of hedges and bands with woody species and wild or herbaceous species or selected species, uncultivated margins, vegetation covers or the preservation of natural areas near the plots allows to increase the diversity of arthropods, birds and other animals controlling agricultural pests (Boller et al., 2004; Franin et al., 2016). The use of green roofs, as an ecological infrastructure capable of housing auxiliary entomofauna useful for the control of citrus pests, is postulated as an independent method of inputs that promotes a more diverse and abundant entomofauna (Ramírez Ferrer, 2019).

The use of hedges provides shelter to the auxiliary fauna population at the end of the crop's vegetative cycle. It is interesting to combine different plant species, with different flowering periods, to obtain a more diversified entomofauna. The hedges, in addition to serving as an accommodation for auxiliary insects, act as a physical shelter reducing the effects of wind, erosive effects, etc.

Vercher Aznar, et al (2008) in their work *"Entomofauna auxiliary associated with natural hedges in Valencian ecological citrus fruits"*, carried out a study on mixed hedges composed of Mediterranean species, such as lentisk, hawthorn, aladierno or cornicabra, and hedges monospecific of cypresses, tree of the sky and pomegranate. The results of this study showed a great diversity of auxiliary fauna, and the species found were similar to those found in citrus fruits, but different from those common in plant coverings. **In both hedges and citrus, coniopterygids (Coniopterygidae) were the most common predators, while in plant cover they were the cecidomids (Cecidomyiidae).** It was also found in this study that the plant species of hedge and the type of plant cover, influences the diversity and abundance of predators found, **being among the hedges the lentisk the species with the highest diversity indexes.** In the results of the first year of this study, **it was observed that coccinellids (Coleoptera) and neuropterans (Neuroptera) constituted 50% of the identified predators, following in importance, ants (17%), spiders (13%), dipterans (syrphid and cecidomids, 11%) and heteroptera (8%). The aladierno species turned out to be a very interesting plant species because it mostly housed insects of the type coccinellids, heteroptera (being the most common family Miridae) and neuroptera (being the *Chrysopa pallens (=septempuntata)* the most common chrysopid (Chrysopidae), especially in the cornicabra). In the case of the cecidomid dipterans (27%), they were present in similar values in all the plant species analyzed in this work.**

Figure 1. Lentisk (*Pistacia lentiscus* L.), plant species used as a hedge, which can house predatory insects with a high diversity index.

As for the flowery bands, these are more attractive than the crop, so that higher levels of pests and their auxiliaries are observed. When choosing the band, species with lots of flowers, pollen producers and with a flowering suitable for the crop cycle should be chosen. As in the hedges, it is interesting to combine different types of species to increase biodiversity.

In other words, in general ecological infrastructures are capable of sustaining populations of natural enemies for various reasons (Silva et al., 2010). First, they provide complementary food sources, such as alternative hosts or preys. Secondly, another important factor is the supply of food sources of plant origin, whether pollen, molasses, floral or extrafloral nectar and even sap and leached sugars that may be important at different stages of the life cycle (Gillespie et al., 2016). Many species of predators and parasitoids have omnivorous feeding. For example, predators of the orders Diptera, Coleoptera, Hemiptera, Thysanoptera, Neuroptera, Lepidoptera and Arachnida take advantage of these resources (Wäckers et al., 2008).

Finally, plant ecological infrastructures offer protection. They generate shelters for estivation or hibernation, favorable microclimates, protection against the wind or against anthropic alterations (Bugg y Pickett, 1998; Boller et al., 2004; Bianchi et al., 2006, Jonsson et al., 2008).

Ramírez Ferrer (2019) in his work *"Auxiliary Entomofauna associated with green roofs on an ecological citrus plot"*, using three types of green roofs: one spontaneous and two sown (***Lolium perenne*** **L**. 50% + ***Trifolium repens*** **L.** 50% / ***Bromus invermis Leyss*** 15% + ***Dactylis glomerata*** **L.** 10% + ***Lolium rigidum*** **Gaudin** 10%, ***Onobrychis viciifolia*** **Mill** 15% + ***Vicia sativa*** **L.** 15% + ***Medicago sativa*** **L.** 15%), monitored the auxiliary entomofauna and the plant composition of the roofs, studying the abundance and diversity of predators, parasitoids and phytophages in each roof; to elucidate whether the variation of the plant composition included in the presence and abundance of the natural enemies of citrus pests. **The results of this study showed that alfalfa (*Medicago sativa* L.) became a dominant cover, staying green even during the warmer months, and presented the greatest overall abundance of all arthropods, including natural enemies. And with respect to predators, the most abundant were the thysanoptera of the genus Aeolotrhips, spiders and cecidomid dipterans and of the genus Platypalpus.**

1.3.1. Aspects and effects of Vegetable Roofs and Hedges in soil management and auxiliary entomofauna.

I. Vegetal Covers

When talking about vegetal covers it is important to know that there is no single type and that, these will vary according to the characteristics of the soil and the interactions that are created with the flora of the place (Domínguez et al., 2010). In a first group, we will talk about adventitious, weed or spontaneous plants in the case of those that appear spontaneously in the field, without the previous action of a farmer for this purpose. In this way, the bad name used until recently: "weeds" is discarded (Guzmán and Alonso, 2008). While, in a second group of this classification, all that flora introduced by the human being will be taken into account voluntarily, to develop specific biological control strategies or with the subsequent objective of mowing or burying it in the area to enrich the soil (Aguilar-Fenollosa et al. 2011; Gómez-Marco et al. 2016). This type of vegetation cover is called green manure or cultivated cover (Domínguez et al., 2010) (Rubio Cebolla, 2016).

The benefits of vegetal covers in general are as follows (Rubio Cebolla, 2016):

- Attraction and protection of auxiliary fauna.
- Protection of the land against erosion and washing.
- Improves the structure of soil aggregates.

- Mobilization of blocked nutrients.
- Nutrient recovery in depth.
- Activation of microbial life (mycorrhizae, symbiotic bacteria).
- Maintenance of moisture and decomposing fauna.
- Return of assimilated nutrients.
- Control of adventitious plants.
- Increase in organic matter and biological activity.
- Soil aeration and increased water retention.
- Ground status indicator.
- Increase the effectiveness of irrigation.
- Evasion of entry into roots and fruits by fungi such as *Phytophthera* spp.
- Allelopathic action.
- Nitrogen retention preventing it from being lost to the atmosphere or by leaching.

There are ideal characteristics for the green cover in the orange tree, and they are the following (Rubio Cebolla, 2016):

- Low growth and superficial rooting, so that it competes as little as possible with trees for water and nutrients.
- Grow well during the rainy season and compete for water with the rest of the adventitious, but not with the crop.
- That does not host pests that cause damage to our crop. That does not interfere with the cultivation operations.
- That is not combustible.
- That is capable of self-seeding.

Among the sown covers, it should be noted for their wide acceptance at the commercial level and for the services recognized in biological control, those composed mostly of grasses. The *Festuca arundinacea* specie adapts perfectly to the conditions of citrus cultivation in the Mediterranean. Studies with this species show that the biological control of the red spider, *Tetranychus urticae*, increases significantly thanks to the fact that on this plant species, develop populations of red spider adapted to monocotyledons, that do not rise to the citrus tree, but allow the appearance of mite complexes abundant and stable predators (phytoseids) with the ability to migrate to the tree when red spider populations exist in it (Aguilar-Fenollosa et al. 2011). Also, the biological control of aphids in citrus fruits is favored by this type of cover (Gómez-Marco et al. 2016).

Covered with flowers (dicotyledonous) can be very useful to offer alternative food resources to natural enemies when they are poorly fed. Recent studies show that the autumn and onset of winter are critical moments in terms of the availability of food resources for many species of natural enemies (Tena et al. 2013). The design of plant covers that offer food at this time will help overcome this food deficit. Numerous species of crucifers such as *Diplotaxis ericoydes* or *Lobularia maritime* are adapted to flourish at this time in conditions of Mediterranean or subtropical climate.

II. Hedges

Guzmán Casado and Alonso Mielgo (2008) define the word and the hedge object as *"rows of trees and shrubs generally located in the limits of the cultivation plots, in the embankments and/or following the course of a stream of water that crosses the farm"* (Rubio Cebolla, 2016).

A hedge can act as a space boundary barrier, either visually or physically (Guzmán and Alonso, 2008; Rubio Onion, 2016).

The hedges must comply the following functions (Rubio Cebolla, 2016):

- They create a refuge zone for the auxiliary entomofauna and provide food during the periods of the year in which the level of plague individuals drops. It is achieved, therefore, that the entomofauna continue in place, avoiding having to replace it again in the future.
- Likewise, hedges also serve as a refuge for pest insects. In this way, the auxiliary entomofauna that is sheltered there, is able to get food. By getting a shelter for both protagonists of the ecosystem, the escape of either is avoided.
- Hedges provide other food sources - such as nectar and pollen - that are essential for most natural enemies. This factor is decisive when choosing the species that will make up the hedge since it will be necessary to alternate its flowering with each other.
- They serve as a refuge for the auxiliary entomofauna in the face of adverse weather conditions.
- They serve as an ecological corridor.
- Act as windbreaker.

The process of obtaining a woody hedge is slow, which is why, herbal species are usually used until the expected result is achieved in a staggered manner. The two types of hedges can be distinguished taking into account the following (Rubio Cebolla, 2016):

- **Herbaceous hedge:** Hedges composed of herbaceous plants are an intermediate step in the conversion of crops to the ecological method. In general, they are more vulnerable plants and modify the microclimate less.

- **Woody hedge:** These hedges must meet certain characteristics to attract natural enemies, and are the following: Early, medium and late flowering (to obtain a continuous flowering), species that produce edible fruits and that are melliferous species.

Table 2. Selection of auxiliary flora and functional hedges to enhance the biodiversity and habitats of beneficial insects or natural enemies.

Natural enemies/ beneficial insects Potentials		List of Auxiliary flora and functional hedges		Target Pest*	References/ Sources
		Vegetal Covers	**Hedges**		
PREDATORS					
Neuroptera	- *Chrysoperla carnea* - *Conwentzia psociformis* - *Crisoperla septempunctata* - *Semidalis aleyrodiformis* - Others species of families: *Coniopterygidae* y *Chrysopidae*	*Medicago sativa* L. (Alfalfa)	*Amaranthus* sp., L. (Moco de pavo, bledo) *Chenopodium álbum*, L. (Cenizo) *Citrus clementina* Hort *Crataegus monogyna* Jacq. *Crithmun maritimun* *Cupressus sempervirens* L. *Ditrichia viscosa* *Efedra fragilis* *Genista umbelata* *Olea europea* – **Plant PP* *Pistacia lentiscus* L. (Lentisk) *Pistacia terebinthus* L. *Rhamnus alaternus* (Aladierno) *Ricino comunis* *Stipa tenacissima* *Thymus hyemalis* *Thymus vulgaris* *Xanthium stramonium*, L. (Cadillo)	-Chinese wax scale (*Ceroplastes sinensis*) - Woolly whitefly (*Aleurothrixus floccosus*) - Citrus leafminer (*Phyllocnistis citrella*) - Nesting whitefly (*Paraleyrodes minei*) - Citrus flower moth (*Prays citri)* - Cotton aphid (*Aphis gossypii*) - Spirea aphid (*Aphis spiraecola*) -*Thrips*	Vercher Aznar, et al (2008) García Marí (2009) Rubio Cebolla (2016) González Fernández (2016) Ramírez Ferrer (2019)

Coleoptera	*Bromus quadripustulatus* *Clitostethus arcuatus* *Chilocorus bipustulatus* *Conwentzia psociformis* *Cryptolaemus montrouzieri* *Delphastus catalinae* *Propylea quatuordecimpunctata* *Rodolia cardinalis* *Rhyzobius lophanthae* *Semidalis aleyrodiformis* *Scymnus interruptus* *Scymnus Subvillosus* *Stethorus punctillum* Species *of coccinellids* Species de *carabids*	*Lolium perenne* L. *Medicago sativa* L. (Alfalfa) *Trifolium repens* L.	*Amaranthus* sp., L. (Moco de pavo, bledo) *Chenopodium álbum*, L. (Cenizo) *Pistacia lentiscus* L. (Lentisco) *Vicia faba* (Habas) *Xanthium stramonium*, L. (Cadillo)	- Red spider mite or two-spotted spider mite (*Tetranychus urticae)* - Chinese wax scale (Ceroplastes sinensis) - Cottony cushion scale (*Icerya purchasi*) - Cotonet de Les Valls (*Delottococcus aberiae*) - Obscure mealybug Cotonet (*Pseudococcus viburni*) - Woolly whitefly (*Aleurothrixus floccosus*) - Mediterranean fruit fly (*Ceratitis capitata*) - Oleander scale (*Aspidiotus nerii*) - California Red scale (*Aonidiella aurantii*) - Cotonet citrus mealybug (*Planococcus citri*) - Cotton aphid (*Aphis gossypii*) - Spirea aphid (*Aphis spiraecola*)	Vercher Aznar, et al (2008) García Marí (2009) Rubio Cebolla (2016) Soler J M (2019) (Bayer) Ramírez Ferrer (2019)

Diptera	*Cecidomyds* *Aphidoletes aphidimyza* *Episyrphus balteatus* *Eupeodes corollae* *Platypalpus flavicornis* *Coenosia attenuata*	*Lolium perenne* L. *Medicago sativa* L. (Alfalfa) *Trifolium repens* L.	*Crithmun maritimun* *Ditrichia viscosa* *Efedra fragilis* *Genista umbelata* *Olea europea* – **Plant PP* *Rhamnus alaternus* (Aladierno) *Ricino comunis* *Stipa tenacissima* *Thymus hyemalis* *Thymus vulgaris*	- Cotton aphid (*Aphis gossypii*) - Spirea aphid (*Aphis spiraecola*)	Vercher Aznar, et al (2008) García Marí (2009) González Fernández (2016) Rubio Cebolla (2016) Ramírez Ferrer (2019)
Heteroptera	**Antocorides:** *Orius, Cardiastethus* *Campyloneura vírgula* *Miridos*	*Medicago sativa* L. (Alfalfa)	*Rhamnus alaternus* (Aladierno)	Lepidopterans Thrips	Vercher Aznar, et al (2008) García Marí (2009) Rubio Cebolla (2016) Ramírez Ferrer (2019)
Thysanoptera	**Genus Aeolotrhips** ***Orius laevigatus*** ***Orius* sp.**	*Lolium perenne* L. *Medicago sativa* L. (Alfalfa) *Trifolium repens* L.	*Chamaemelum mixtum*, L. *Vicia faba* (Habas) *Whytania frutescens* **Plants that offer alternative prey to *Orius* sp.:** *Thymelaea hirsuta* – **Plant PP* *Thymus hyemalis*	Lepidopterans (larvaes) Thrips	Rubio Cebolla (2016) González Fernández (2016) Ramírez Ferrer (2019)

Hemiptera	**Nabis** **(Hemiptera: Nabidae)**		*Crithmun maritimun* *Ditrichia viscosa* *Efedra fragilis* *Genista umbelata* *Olea europea* – **Plant PP* *Ricino comunis* *Stipa tenacissima* *Thymus hyemalis* *Thymus vulgaris*	Lepidopterans	González Fernández (2016) Rubio Cebolla (2016) Ramírez Ferrer (2019)
Diptera: Syrphids	**Syrphid flies**		*Amaranthus* sp., L. (Moco de pavo, bledo) *Brassica campestris* M (Colinabo) *Chenopodium álbum* L. (Cenizo) *Vicia faba* (Habas) *Xanthium stramonium*, L. (Cadillo)	-Citrus flower moth (Prays citri) - Cotton aphid (*Aphis gossypii*) - Spirea aphid (*Aphis spiraecola*) -*Thrips*	Rubio Cebolla (2016) González Fernández (2016)
Mites	**Phytoseiid mites** *Euseius stipulatus* *Neoseiulus californicus Phytoseiulus persimilis Typhlodromus phialatus*	*Lolium perenne* L. *Trifolium repens* L.	*Celtis australis.* *Rhamnus alaternus* *Viburnum tinus*	-Citrus bud mite *(Aceria sheldoni)* - Citrus Red Mite (*Panonychus citri*) - Spider mites Genus *Eutetranychus* (*E. orientalis* & *E. banksi*) - Red spider mite or two-spotted spider mite (*Tetranychus urticae*) - Nesting whitefly (*Paraleyrodes minei)* -Thrips	García Marí (2009) González Fernández (2016) Rubio Cebolla (2016)

Spiders	*Icius hamatus*	*Lolium perenne* L. *Trifolium repens* L.	*Chamaemelum nobile* (Manzanilla) *Crithmun maritimun* *Ditrichia viscosa* *Efedra fragilis* *Genista umbelata* *Olea europea* – **Plant PP* *Ricino comunis* *Symphytum officinale* (consuelda) *Stipa tenacissima* *Thymus hyemalis* *Thymus vulgaris*	Lepidopterans Mediterranean fruit fly	García Marí (2009) Rubio Cebolla (2016)
Other spiders	*Phylodromus cespitum* *Cheiracanthium mildei* *Olios argelasius* *Clubiona genevensis*		*Chamaemelum nobile* (Manzanilla) *Symphytum officinale* (consuelda)	Mediterranean fruit fly	García Marí (2009) Rubio Cebolla (2016)

<table>
<tr><th colspan="6">PARASITOIDS</th></tr>
<tr>
<td rowspan="2">Hymenoptera</td>
<td>Aphelinidos
Hymenoptera: Aphelinidae</td>
<td></td>
<td>Ditrichia viscosa
Doricnium pentaphillum
Efedra fragilis
Ricino communis
Rosmarinus officinalis
Stipa tenacissima
Thymus hyemalis
Thymus vulgaris
Viburnum tinus.
Whytania frutescens</td>
<td rowspan="2">-Chinese wax scale (Ceroplastes sinensis)
- Smaller green leafhopper (Empoasca)
- Obscure mealybug Cotonet (Pseudococcus viburni)
- Woolly whitefly (Aleurothrixus floccosus)
- Oleander scale (Aspidiotus nerii)
-California Red scale (Aonidiella aurantii)
- Cotonet citrus mealybug (Planococcus citri)</td>
<td>González Fernández (2016)

Ramírez Ferrer (2019)</td>
</tr>
<tr>
<td>Encyrtidos
Hymenoptera: Encyrtidae
Hymenoptera: Braconidae (Apanteles sp.)</td>
<td></td>
<td>Convolvus sp
Ditrichia viscosa
Poligonum sp., L.
Ricino communis
Stipa tenacissima.
Thymus vulgaris</td>
<td>González Fernández (2016)
Ramírez Ferrer (2019)</td>
</tr>
<tr>
<td colspan="2">Ichneumonid parasitoids (Hymenoptera)-parasitoid wasps

Parasitoids family flies Tachininae (Diptera)</td>
<td></td>
<td>Umbeliferous plant hedges (familiy Apiaciae)</td>
<td>Lepidopterans</td>
<td>Ramírez Ferrer (2019)</td>
</tr>
<tr>
<td colspan="2">Interesting plant species for the design of HEDGES because they are producers of pollen and/ or nectar that offer food to general predators</td>
<td></td>
<td>Phyllirea angustifolia – *Plan PP
Thymelaea hirsuta – *Plant PP
Olea europea – *Plant PP</td>
<td></td>
<td>González Fernández (2016)</td>
</tr>
</table>

* Pollen Production Plant: Planta

Vegetal Covers		
 *Medicago sativa* L. (Alfalfa)	 *Lolium perenne* L.	 *Trifolium repens* L.
Hedges		
 *Amaranthus* sp., L. (Moco de pavo, bledo)	 *Thymelaea hirsuta*	 *Vicia faba* (Habas)
 *Xanthium stramonium*, L. (Cadillo)	 *Thymus vulgaris* (tomillo)	 *Chenopodium álbum*, L. (Cenizo)

Figure 2. Selection of auxiliary flora and functional hedges to enhance the biodiversity and habitats of beneficial insects or natural enemies (See table 2). Source of images: http://herbarivirtual.uib.es/ca/general/ , https://es.m.wikipedia.org/

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5. Protocol to determine and improve soil health in citrus cultivation

Project title: Development of sustainable control strategies for citric under threat of climate change & preventing entry of HLB in EU

Protocol to determine and improve soil health in citrus cultivation"

1.- INTRODUCTION

Soil health is defined as soil quality, that is, it is the soil continuous ability to function as a vital ecosystem that supports plants, animals and humans (USDA-NTCS, 2012). As is known, the soil is a natural corp of great importance that provides environmental services, allows the growth of plants, stores water and retains nutrients, it is also a reservoir of organisms such as bacteria, fungi, nematodes, etc. In order to make the soil works correctly, it is important to use management practices aimed at improving soil health and achieving agronomic (increasing productivity and crop profitability) and environmental, immediate and future benefits.

From an agricultural and environmental point of view, soil health is directly related to favorable physical, chemical and biological properties that promote the plants development and help with environmental quality. This concept can be divided into *physical health* of soil, *chemical health* of soil and *biological health* of soil; which we will define below.

- *Soil physical health*. This concept relates to the balance that soil has in preserving and draining water, as well as its ability not to restrict the plant root growth. ~~The~~ Aabove is related to soil texture, permeability, porosity and drainage, mainly.

- *Soil chemical health*. It is defined as capacity of the soil so that nutrients are in balance and available to plants and soil acidity and alkalinity are in an optimal range for the crop, and there are not salinity or sodicity problems.

- *Soil biological health*. Soil biological properties mainly refer to the existence and action of organisms such as bacteria, fungi, nematodes, earthworms, annelids and arthropods (Fertilab), and their evaluations are related to the decomposition of organic material derived from plant and animal waste, as well as its recycling, since by-products of its action directly influence the physical and chemical properties of soils (Astier, et al., 2002). This property is so dynamic that it allows monitoring degradation or recovery soil progress.

Soil biological propertiesinclude wealth and abundance of wildlife , microbial biomas carbon and nitrogen, the potentially mineralizable nitrogen and the soil respiration (Doran, et al. 1994), the last one is the parameter most related to soil health. . The biological indicators mentioned are part of soil biological and chemical properties, which allow interpreting organic material dynamic and organic waste processes of transformation. Soil biological parameters are sensitive to environmental stress, respond quickly to changes in soil management and are easy to measure.

2.- MEASUREMENT OF SOIL QUALITY

Physical indicators to be determined

- ***Soil texture:***

Soil is mainly composed by minerals (45%), water and air (50%) and organic material (5%). Soil texture refers to the content and proportion of the mineral particles of sand, silt and clay in the soil. Soil texture is a characteristic of great relevance; influences fertility, water storage (water retention), permeability (water drainage) and soil aeration.Soil texture issoil texture

Soil particles properties :

Properties	***Sandy soil***	***Clay soil***
Nutrients	*Little bit*	*Much*
Aeration	*Good*	*Bad*
Permeability	*High*	*Very little*
Water storage	*Little bit*	*Much*

Source: González, 2015

- ***Organic material:***

Organic material is a very important factor for soil health, necessary to maintain the nutrients available for cultivation and soil organisms, retain soil moisture, allow easy handling and reduce the incidence of diseases and blights in crops . Most affected organisms in the soil live linked to soil organic material, being necessary for development of important activities that they carry out for conservation of soil health. Thus, most microorganisms are found in soil organic part .

Chemical indicators to be determined

- ***Soil nutrients:***

Soil must present essential nutrients (macro and micro-nutrients) for optimal growth and profitability of the crop. In the availability of nutrients, several characteristic aspects of the soil are involved, such as texture, organic material content and micro-organisms (wildlife) present in soil (bacteria, fungi, nematodes and earthworms).

- **Soil pH:**

Soil pH, a measure of acidity or alkalinity, affects the availability of soil nutrients and determines solubility of minerals present, as well as microorganisms activity.

So, most minerals are more soluble in acidic soils than in neutral or slightly basic soils. The highest availability for most nutrients is in a pH range between 6.0 and 7.0.

Soil pH also affects microorganisms activity beneficial to soil and crop, indirectly affecting nutrients availability.

In general, while fungi have a wide optimal pH range, bacteria and actinomycetes work best at intermediate or relatively high pH.

- ***Salinity:***

The electrical conductivity (EC) of the soil solution indicates the amount of salts present. All soils require some salts, essential for crop growth, an excess of salts can inhibit growth and crop production.

Although soil salinity may be due to natural causes, this is often due to improper use and management of the soil, such as excess fertilizers or the use of saline waters for irrigation.Saline soils are usually found in arid and semi-arid regions, where low annual precipitation does not allow to wash salts and accumulate these in the soil profile.

Electrical conductivity (dS/m or mmhos/cm), measures amount of cations or anions (salts) present in the soil solution. Ions generally associated with soil salinity are Ca ++, Mg ++, K +, Na +, H + (cations) and NO3-, SO4-, Cl-, HCO3-, OH- (anions).

Soil salinityl influences the availability of water for the crop, making decreasetheir growth and yield. The presence of salts hinders ability of the roots to absorb water due to increased soil solution osmotic potential.

On the other hand, some anions and cations produce problems of toxicity in plants and / or problems of permeability in soils, like a Cl- and Na +, both toxic to citrus cultivation, and Na + as dispersant of clay particles, which alters soil structure making drecrease its infiltration capacity and hydraulic conductivity.

In general, at a salinity of 1.71 dS/m symptoms begin to appear at the level of crops and microbial wildlife, making decrease crop yields and altering soil microbial processes. However, each crop has a certain tolerance to salts, for example, citrus cultivation is sensitive to salinity with yield reductions from 0.9 dS/m.

Biological indicators to be determined

Microorganisms contribute to the maintenance of chemical, physical and biological soil fertility. They transform inorganic nutrients, which otherwise cannot be absorbed by the plant; They also favor the decomposition and mineralization of organic material. Life in the soil is made up of microorganisms such as bacteria, fungi, protozoa, nematodes, viruses and algae; and macroorganisms in soils include vertebrates and invertebrates. This last group includes arthropods that vary from mites to large beetles, millipedes, termites and earthworms, among others.

Thus, the indicators to be determined in laboratories will be:

- **Microorganism count (cfu/g*)**

**cfu/g: Colony forming units of bacteria per gram of soil*

  - **Bacteria.** Bacteria break down easy-to-use substrates, simple carbon compounds such as radical exudates and fresh plant residues. Waste produced by bacteria becomes organic material. Some microorganisms can break down pesticides and pollutants in the soil. They are especially important in the immobilization and retention of nutrients in their cells and, therefore, prevent the loss of nutrients from the root zone. *Mesophilic Bacteria, Thermophilic Bacteria and Nitrogen Transforming Bacteria* will be determined in laboratory.

  - **Fungus**. They break down the most resistant organic material, retaining in soil the nutrients obtained in the form of fungus biomass and CO2 release. First, the less resistant material is broken down while the more resistant material, such as lignin and proteins, breaks down into various stages. Many of the secondary waste products are organic acids; therefore, fungus help increase the accumulation of organic material rich in humic acids, resistant to further degradation. There are those of free life and others like mycorrhizae that live in symbiotic form in the roots of plants. In addition to beneficial fungus, there are also phytopathogens. Therefore, the indicators to be determined in laboratory will be *Molds and Phytophthora spp*.

  - **Nematodes**. These organisms not only weaken plants and decrease yields by their direct action on the roots, they also act in etiological complexes that involve fungi, bacteria and virus. When there are nematodes that feed on bacteria and fungus, nitrogen is released as (NH4+)), nitrogen that is available for the growth of plants and other soil organisms.

- **Number of earthworms**. They promote microbiological activity by means of fragmentation of the organic material and the increase in the area accessible to fungus and bacteria. Into the bargain, they stimulate extensive root growth in the subsoil due to the greater availability of nitrogen in the tunnels and the easy penetration of the roots through existing channels

Sampling for determinate biological indicators

Each plot will be divided into different sampling units based on soil heterogeneity (color, soil drainage, slope ...) and plantation (productivity, growth ...).

From each sampling unit we will take a sample of the surface layer (soil: 0-30 cm depth) and another of the immediately lower layer (subsoil: 30-60 cm depth). Each of these samples will be composed of several subsamples, with a number of 10 to 15 subsamples being recommended, which will be taken at random. Each sample will weigh approximately 1kg.

The extraction of the ground will be carried out with an auger in a wet bulb area. Each extracted subsample will be collected in a plastic bag, eliminating any foreign objects (stones, roots ...), where they will be mixed and the necessary sample will be taken (approximately 1 kg).

Soil samples will be taken preferably after harvest and never after applying a fertilizer or amendment.

3.- INTERPRETATION OF THE RESULTS

Healthy soil increases production, profitability and protects natural resources (air and water). To determine if a soil is healthy, its physical, chemical and biological characteristics must be evaluated.

HEALTHY SOILS	UNHEALTHY SOILS
Physical aspects	
Top layer of deep soil with presence of organic material	Eroded surface layer without organic material
Good drainage	Compacted / flooded
Water storage capacity	Poor (runoff) or excessive drainage (deep leaching)
Chemical aspects:	
Good balance and nutritional status of the soil	Imbalance and / or nutrient deficiency
Suitable pH	Unsuitable pH
Low salinity and absence of toxic elements	High salinity and presence of toxic elements
Biological aspects:	
High presence of worms	No worms

High beneficial microbial activity	Exhibits pathogenic microorganisms or absence of microorganisms
Other observations:	
Easy to work	Difficult to work
Nice smell*	Unrelated smell
Abundant non-invasive cover crops	Naked soil

Source: González, 2015

*Healthy soils have a sweet and pleasant smell of ground, while an unhealthy soil has a sour or metallic smell

4.- PRACTICES TO IMPROVE SOIL QUALITY

In the case of unhealthy soils, and therefore of low productivity, practices should be used to improve soil quality, aimed at:

- Pay attention to soil fertility
- Take care and increase the life of it
- Prevent soil erosion
- Establish soil conservation practices

Among these practices, in citrus cultivation, can be considered:

- Minimum tillage and establishment of green roofs in the streets of the plantation.

  The cover crops on the streets of the plantation increases the organic matter of the soil, prevents its erosion, conserves its humidity, increases the nutrient cycle, provides nitrogen for the crop, controls weeds and reduces soil compaction.

- Improvements in drainage: use of a ridge, incorporation of organic matter, black net mulch of plant lines...

  The use of black net (polypropulene) mulch of the plant lines, allows to reduce soil erosion, moderate its temperature and air quality, increase organic matter, conserve moisture and control the proliferation of weeds.

- Management of chemical and organic waste: efficient application of phytosanitary products (avoiding drifts), incorporation of pruning remains in the soil (crushed and buried) …

- Integrated pest management.

  Perform an integrated pest management in order to minimize the use of pesticides through cultural and biological practices. The use of pesticides can affect living organisms that are in the soil, which are necessary to break down organic matter and increase the availability of nutrients for the crop.

- Water conservation: irrigation installation and efficient irrigation water management, application of washing fraction in case of presence of salts…

- Efficient and balanced fertilization, avoiding antagonisms between elements.

  It is important to apply the nutrients in the appropriate amounts, as well as at the physiologically required time by the crop and under favorable environmental conditions. In addition, the dose of fertilizers must be adjusted (corrected) based on the foliar analysis (nutritional status of the tree) and the irrigation water (nutrient supply through irrigation), thereby allowing a better utilization of nutrients by cultivation, reduce operation costs and chemical residues on the ground and pollution of aquifers, thus maintaining the quality of water and soil.

- Use of organic fertilizer

The realization of these practices translates into an improvement in crop production and conservation, water quality and efficiency, as well as a reduction in the use of fertilizers and pesticides.

Thus, within the practices of improvement of soil quality more interesting for the cultivation of citrus, and also for the health of the plant, the following are identified:

4.1.- Black net mulch of the plant lines (ridge):

Currently, the black net mulch of the citrus plant lines is presented as an interesting technique from an environmental and agronomic point of view.

As for the advantages over natural resources, the use of black net mulch of the plant lines contributes to both soil conservation and the most efficient use of irrigation water, allowing water savings of 30%. Thus, its use allows an effective control of the adventitious plants of the plantation line and a considerable saving in the irrigation water as a result of the reduction of the direct evaporation of the water of the soil and of the less competition for the water with the plants Adventures of the plantation line (Romero-Rodríguez et al., 2016). The mechanical control of the black net mulch on the adventitious plants replaces the use of herbicides, minimizing the contamination produced by them.

As for the agronomic or tree-level advantages, the black net mulch of the plant lines induces greater tree growth, an advance in the entry into production (Hervalejo et al. 2012; Romero-Rodríguez et al., 2013) and an improvement in the internal quality of the fruit. It also means protection against various phytosanitary problems, reducing the risk of root asphyxiation in situations of heavy rainfall, as well as the incidence of watery fruits by avoiding direct splashing of the soil.

As for inorganic mulch, black net mulch is more sustainable in the long term, generating a smaller volume of agricultural waste than others such as plastic. Thus, the technical characteristics of this net, 6 x 4.5 polypropylene, of great durability (between 8 and 10 years) together with its main interest in use in citrus plantations, which resides in the first years of planting until the tree reaches a sufficient canopy size to optimally shade the planting line, it makes a single installation (at the beginning of planting) sufficient.

Photograph 1.- Detail of black net mulch of citrus plant lines

Installation details:

Black net mulch is done only on plant lines. Before its colocation, ridge must be prepared. Then, two grooves are made at the base of the ridge, one on each side of it, being able to use a manual implement or a fence.

Photograph 2.- Making side grooves on a ridge of the

Photograph 3.- Spreading and fixing black net

A black net piece is then spreaded along each of the two grooves made, both blacn net pieces are extended to the center of the ridge and overlapped each other by at least 15 cm. The black net is secured of the following manner: the edges of the black net located on both sides of the plant line (ridge) are covered with the soil previously removed from the grooves, while the ends of the black net overlapped on the center of the plant line (ridge) are fixed each other with plastic clips or with metal forks nailed to the ground.

Photograph 4.- Fixing the two black net pieces over the center of the ridge

In the case of new plantations, the black net can also be extended in a single piece. Once the black net is fixed on both sides of the ridge (at its base) the necessary cuts will be made in the center of the ridge to introduce the seedling.

Photograph 5.- Spreading of the black net over the ridge

Photograph 6.- Black net cuts and planting of the seedleing

In the irrigation system, the dripperlines can be placed in two ways: under the black net, easier and cheaper action, with easy access to the drippers in the case of their clogging (opening the both net black pieces superimposed on the center of the ridge); or above the black net, installation with more dripper shutter problems and more expensive. In the event that it is chosen for its placement above the black net, the carrier branch is placed above the balck net from which micro-tubes are inserted into the net through a small hole that is made in this.

As the only aspect to take into account, highlight the importance of good maintenance of irrigation facilities, especially in regard to cleaning drippers in order to avoid clogging.

4.2.- Irrigation strategies: efficiency of use and improvement of water quality

The impact of climate change threatens the Mediterranean Basin with a future scenario of lower availability and water quality as a result of increased temperatures, uncertainty in precipitation, more scarce and concentrated over time. This situation, together with the increase in the demand for food in 2050 (FAO, 2017), 50% higher than in 2012, as a result of the increase in the world population (9.7 billion people), will result in greater pressure on natural resources, thereby aggravating the effects of climate change. This situation requires sustainable strategies in the use of irrigation water.

It should not be forgotten that in many citrus regions of the Mediterranean Basin, water demand is currently well above the available resources, the search for strategies that allow a more efficient use of water in agriculture is necessary (sustainable deficit irrigation, controlled deficit irrigation), as well as greater availability of water for irrigation (use of low quality water).

4.2.1.- Deficit irrigation

Deficit irrigation (DI) is considered as an alternative aimed at improving water use efficiency, increasing its productivity (kg/m^3 of water). Through deficit irrigation it is intended to minimize water expenditure without significantly affecting the final crop production.

There are different deficit irrigation strategies based on the way to perform the water cut (García-Tejero et al., 2010):

• Sustained deficit irrigation (SDI) takes place a water cut (on the water needs or evapotranspiration of the crop: ETc) constant throughout the irrigation campaign.

• Low frequency deficit irrigation (LFDI) consists of the alternation (cycles) of periods of irrigation and periods of restriction depending on the hydric state of the crop, establishing thresholds of water potential in stems at midday (Ψt) within the which should be maintained.

Culture evapotranspiration (ETc) is calculated through the product between potential evapotranspiration (ET_0), according to Allen et al. (1998), and a culture coefficient (Kc) and reduction (Kr) according to the methodology described by Doorenbos and Pruitt (1974).

According to work carried out in trials with different citrus deficit irrigation strategies (García-Tejero et al., 2010) the following irrigation strategy is established:

Sustained deficit irrigation (SDI) with a water cut of 30% of the crop's needs (ETC) in years with optimal rainfall records during the spring (PhI: flowering and fruit set), applying an irrigation during this period (PhI) Low frequency deficit (LFDI) taking into account a threshold of Ψt of stem at midday between -1.3 and -1.5MPa. These strategies allow a water saving between 20 and 35%, as well as an improvement of the internal quality of the fruit (increase in the sugar content of the juice; ºBrix).

4.2.2.- Controlled deficit irrigation

Controlled deficit irrigation (CDI) is a water saving strategy in which water restrictions are made considering the development phase in which the crop is located, which have a different sensitivity to water scarcity, thereby minimizing its impact on the production and quality of fruit.

In relation to the phenological phases of the crop, considered for the application of controlled deficit irrigation (CDI), three different stages are established:

- PhI: Flowering and setting, from mid-March to the end of June approximately.

- PhII: Fruit growth, from late June to early September.

- PhIII: Fruit ripening, from the beginning of September until the fruits are harvested.

According to work carried out in trials of controlled deficit irrigation in citrus fruits (García-Tejero et al., 2010; Ginestar and Castel, 1996; González-Altozano and Castel, 1999; González-Altozano and Castel, 2000) the following irrigation strategy is established according to the cultivated variety:

I.- `Clementina de Nules´ (mandarins): it is the flowering and fruit set (PhI) phenological phase the most sensitive to the water deficit, establishing the phenological phase of fruit growth (PhII) the most suitable for the restriction of irrigation water . The recommended controlled deficit irrigation strategy would be to apply a water restriction between 30 and 50% of the ETc during the PhII of fruit growth (end of June to beginning of September) which can lead to savings of Water up to 20%.

II.- `Salustiana´ and` Navelina´ (oranges): the flowering and fruit setting (PhI) and fruit growth (PhII) phases are the most sensitive to the water deficit, establishing the phenological phase of fruit ripening (PhIII) the most suitable for the establishment of the irrigation restriction. The most advisable strategy is the reduction of 45% of the ETc during the PhIII (from the beginning of September until the collection) with reductions of 30% of the ETc during the phenological phases PhI (from mid-March to the end of June) and PhII (end of June to beginning of September). This strategy allows a water saving of approximately 30%.

III.- `Fino´ (lemon): it is growth phase of the fruit (PhII) the most sensitive to the water deficit, on the contrary according to works in` Fino´ the phenological phases of flowering and fruit set (PhI) as well as The phenological phase of ripening of the fruit (PhIII) is suitable for the imposition of the irrigation restriction (Domingo et al., 1996). However, given the sensitivity of the phenological phase of flowering and fruit set in other citrus species (PhI), it is proposed to recommend an intermediate strategy consisting of a reduction of 45% of the ETc during the PhIII (from the beginning of September until the collection) with reductions of 30% of the ETc during the phenological phase PhI (from mid-March to the end of June) and the application of 100% of the ETc in the phenological phase PhII (end of June until the beginning of September). This strategy allows a water saving of approximately 20%.

For the success of these DRC strategies, a Ψt of stem at midday in summer from -1.2 to -1.3 MPa is recommended.

The CDI treatments increase the total soluble solids (sugars; ºBrix) and the acidity of the fruit juice, which represents an economic advantage for the citrus markets.

4.3.- Cover crops in the citrus row middles

The control of soil water erosion requires adequate techniques for its management and conservation. Planting cover crops is considered as one of the best measures to prevent soil erosion, improve its structure and infiltration, minimize pollution caused by the use of phytosanitary products (Sands -Arenas et al., 2015), provide organic material, promoting enzymatic and microbial activity, and increasing the availability of nutrients for the crop, and favoring biological control.

The most suitable cover crops for citrus are (Arenas-Arenas et al., 2015):

Annual species:

- Vicia sativa (Veza; 100 kg/ha) + Avena sativa (Avena; 80 kg/ha).
- Veza (60 kg/ha) + Pisum sativum (Guisante; 70 kg/ha) + avena (70 kg/ha).
- Vicia ervilia (Yeros; 60 kg/ha) + Lolium rigidum o Lolium multiflorum (Vallico; 25 kg/ha) + Melilotus officinalis (Meliloto; 25 kg/ha).

Perennial Species:

- Trifolium repens (Trébol blanco; 8 kg/ha) + Medicago sativa (alfalfa; 25 kg/ha) + Lolium perenne (ray-grass inglés; 25 kg/ha) o Festuca arundinacea (festuca; 40 kg/ha).

- Ray-grass inglés (25 kg/ha) o festuca (40 kg/ha) + Medicago spp (Mielgas; 10 kg/ha).
- Trifolium repens, Trifolium pratense o Trifolium subterraneum (Trébol blanco o morado; 10 kg/ha) + Dichondra (20 kg/ha) para zonas sombreadas.

Cover crops can provide large amounts of humus and nutrients annually (Domínguez-Gento et al., 2003). Thus, for example, a cover composed of a vetch or an alfalfa can provide more than 100 kg of nitrogen per hectare, if after its harvest it is incorporated into the land.

Planting cover crops takes place at the beginning of autumn, for which a seed bed should be prepared with a little work. After you let it grow throughout the winter, you can mow the cover once the spring has begun and it is in competition with the plantation (end of March).

Photograph 7.- Detail of a cover crop in the citrus row middle

4.4.- Improvement of biological control:

4.4.1.- Hedges

Biodiversity is a basic pillar for the maintenance of fertility and crop health. Flowering plants below or near the crop will maintain a wide range of parasites and predators that will find alternative food and breeding areas in those, making a natural control of unwanted insects.

Live hedges serve as a natural barrier, isolating the crop from environmental contaminants. They will also serve as shelter and food for auxiliary fauna. The control of aphids and other phytophages is closely related to these wild species.

The choice of species in a hedge must be carried out by different criteria:

- Adaptation to the environment. Association
- Fast and adequate growth with the environment.
- Low competition with the crop.
- Low or no invasive potential.
- Abundant flowering and complementary to the crop.
- Low maintenance and adaptation to strong pruning.
- 50% wind permeability.
- Contribution of alternative uses or useful by-products.

The plants used as a hedge of better growth in the citrus environment are the Mediterranean species that belong to a natural plant community, if possible evergreen, to choose according to the type of microsystem where the plantation is located:

For loose soils:

- *Pistácea lentiscus*
- *Rhamnus alaternus*
- *Phyllirea angustifolia*
- *Viburnum tinus*
- *Fraxinus ornus*
- *Pistacea terebinthus*
- *Arbutus unedo*
- *Myrtus communis*
- *Genista* spp., *Retama* spp.,…

For *heavy soils*:

- *Nerium oleander*
- *Salix* spp.
- *Pistácea lentiscus*
- *Rhamnus alaternus*

For warm climate or close to the sea:

- *Nerium oleander*
- *Pistácea lentiscus*
- *Laurus nobilis*
- *Tamarix* spp.

4.4.2.- Release of natural enemies

An interesting method to manage or lower populations of certain problematic arthropods is **biological control**. In addition to the establishment of flora that serves as a refuge for predators and parasitoids, through the use of plant coverings and hedges, the inoculative or massive release of natural enemies for certain pests, and at certain times, can be interesting.

There are different commercial houses that offer species of insects or microorganisms useful in biological control, admitted by the European Regulation. Likewise, there are insectaries of the Plant Health Services (such as Silla in Valencia and Almanzora in Almería) in which the following arthropods can be found according to the pest to be controlled:

- Citrus melibug (*Planococcus citri*):
    - Cryptolaemus montrouzieri
    - *Anagyrus*
- Red scale (*Aonidiella aurantii*):
    - Aphytis melinus
    - *Encarsia*
    - *Comperiella bifasciata*
      Rizobius lophantae
- Cottony cushion scale (*Icerya purchasi*)
    - *Rodolia cardinalis*
- *Ceratitis capitata, Bractocera oleae*:
    - *Opius concolortae*

4.4.3.- Hotels for insects

Insect hotels are man-made artificial structures to provide shelter for beneficial insects during the summer and hibernation seasons. In general, insect hotels favor the ecosystem, pollination and biodiversity, creating an environment that favors the balance between a large number of botanical species and a large number of animal species. Hotels attract predatory insects that help control citrus pests. A protocol for the installation and maintenance of insect hotels and nest boxes has been carried out within the framework of this project

Although the full content of this protocol must be taken into account, this document extracts the final part of it in which the following key aspects are established as a summary or conclusion (Teh-Weisenburger, 2019):

• Be realistic: a small hotel is better than large. Evaluate the area where you plan to establish your hotel or insect shelter. Think small and have several units that house a species instead of a single large unit that attempts to house an entire zoo, which requires potentially conflicting environments.

• Choose a responsible design. There are a number of good online guides for hotels written by entomologists. Melanie von Orlow (2019) has written a book with detailed manuals, available in Dutch and German.

• Build it well: with materials such as natural wood, untreated and without chemicals such as varnish, paint or wood protector that repel insects. The construction material will be treated with natural waterproofing substances such as virgin wax or oils, avoiding those that generate insect rejection. To promote sustainability, consider the use of recycled or natural materials. Good insect hotels should be built sturdy with a solid back and roof / shelter to protect from rain. The shelter must be well insulated and protected from moisture.

• Install it well: For example, bee hotels should be located in full sun, facing southeast or south, at least one meter from the ground, with no vegetation in front of it obscuring the entrances to the tunnels. It must also be fixed securely to prevent it from shaking and swaying in the wind.

• Include other important habitat resources to attract a variety of beneficial insects, such as: flowering plants that can feed insects, a water source, wintering sites and wind shelter, etc. (Photograph 8)

Photograph 8.- Insect hotel with other important habitat resources: flowering plants, protection against the wind ... Image source: Experimental area of organic farming in ICIA (Valle de Guerra, Tenerife).

• Maintain and clean the hotels. This is the most overlooked part in regards to an insect hotel. Taking care of the insect hotel is very important. For example, bee hotels should be inspected at the end of the summer to remove and clean dead cells. Cleaning will prevent mold and mites that would multiply on dead bees or larvae. Without timely maintenance and cleaning, a once-occupied insect hotel may not attract a new lot next season.

• Replace when the time is right: Insect hotels can degrade naturally after two or more years since the material used is not treated. Change the nesting blocks or pieces every two years to avoid the accumulation of mold, mites and parasites over time

4.5.- Sustainable fertilization:

Soil microorganisms have an important role in the improvement and maintenance of fertility, transforming inorganic nutrients and favoring the decomposition and mineralization of organic material.

Long periods of monoculture, such as citrus, can reduce soil quality in terms of microbial diversity and community structure. However, certain practices such as the use of organic fertilizers, biostimulants, slow-release mineral fertilizers and strains of mycorrhizae and trichodermas improve the microbiological quality of the soil and its fertility, thereby allowing a more sustainable fertilization of the crop.

4.5.1.- Mycorrhizae

Mycorrhizae are fungi that establish a symbiosis relationship with most plants, providing better nutrition, stress tolerance and balanced growth. In exchange for a small amount of carbohydrates, mycorrhiza gives the plant fundamental nutrients and water at the most critical moments of its development.

The benefits include the best tolerance to abiotic stress, which includes episodes of drought, salinity and extreme temperatures. It facilitates the uptake of minerals, especially phosphorus, zinc and copper, and resistance to adverse soil pH.

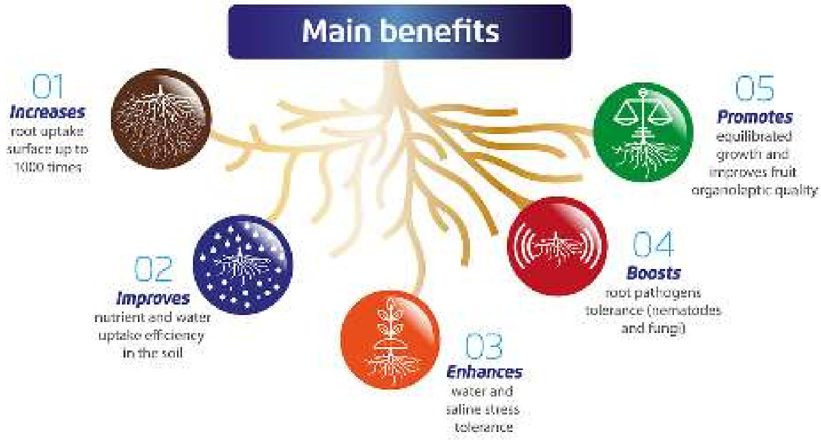

Figure 1.- Main benefits of mycorrhizae in plants
https://www.atens.es/es/microorganismos/micorrizas/

The incorporation of mycorrhizae to the cultivation of citrus fruits increases the root system of the plant, and therefore, the absorption capacity of nutrients and water. In addition, it provides citrus fruits with greater resistance to any type of biotic or abiotic stress, favoring a balanced crop development. It quickly colonizes the soil and the rhizosphere of the plant leaving no other beneficial fungi without resources and spaces. Fortifies the plant acting at the molecular level.

Among mycorrhizae, *Rhizoglomus irregularulare* BEG72 (formerly *Rhizophagus intraradices*) is one of the best known and studied species. There is an extensive scientific literature that evaluates their abilities to promote plant growth and crop versatility. It has a very dynamic growth and ensures efficient absorption of nutrients from the soil. The BEG72 strain was isolated by the Institute of Agrifood Research and Technology (IRTA, Spain), an applied research institute of reference in agronomy.

Different commercial houses have products of this kind, such is the case of ATENS.

4.5.2.- Trichodermas

Trichoderma spp. are fungi of free life present in several soils and root ecosystems. They are symbionts of opportunistic and avirulent plants. These root-microorganism associations lead to changes in the plant's metabolome, which improves root growth and development, crop productivity and nutrient absorption and use.

Atens has investigated *Trichoderma koningii* TK7 for several years to find a strain that can effectively colonize the roots and form a symbiosis. TK7 competes for key seed exudates or plant roots that stimulate the germination of plant fungal propagules in the soil and, more generally, compete with soil microorganisms for nutrients and / or space. TK7 also increases the absorption and concentration of a variety of nutrients (phosphorus, iron, manganese) and can synthesize plant hormones. The use of *Trichoderma* ensures stronger roots for citrus crops.

4.5.3.- Biostimulants

There are products based on plant extracts that promote plant metabolism and cell division, improve plant growth and development and increase plant resistance to climatic conditions and unfavorable diseases.

There are scientific studies that support the benefits of these products on crops, such is the case of HEPTABIOL from the ATENS commercial house on melon cultivation (Lucini et al., 2018). Radical biostimulant from 7 plant extracts that acts by modulating the hormonal metabolism of the plant. Among the benefits provided by HEPTABIOL can be mentioned:

- Stimulates radical growth.
- Protects against water stress.
- Increases antioxidant activity.
- Improves the response to biotic stress
- Promotes flowering and ripening of the fruit due to its auxin content.
- Stimulates the growth of mycorrhizae and *Trichodermas*.

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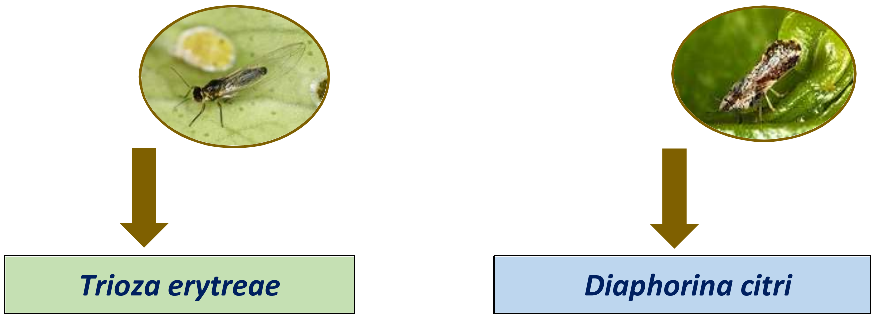
6. Alternative Natural Vector Control Solution

Project title: Development of sustainable control strategies for citric under threat of climate change & preventing entry of HLB in EU

3. Protocols for Production Efficiency
3.2. Alternative Natural Vector Control Solution



- 3.2. Alternative natural vector control solution
  - 3.2.1. Chemical control strategies
  - 3.2.2. Phytosanitary Control in Ecological Citriculture
  - 3.2.3. Biorational's products to control of psyllids in citrus
  - 3.2.4. Biological control of psyllids
  - 3.2.5. Entomophatogenic fungi to control of psyllids
- References

3.2. Alternative Natural Vector Control solution
3.2.1. Chemical control strategies

The chemical management programs of the HLB vectors must apply the principles of Integrated Citrus Management, seeking effective control of the vector but at the same time, preserving as much as possible the benefitial fauna associtated to the crop (Monzó et al., 2014) and reducing to the maximum the risks of development of resistance to the active ingredients used (Tiwari et al., 2011). For this, those products that are effective in the control and respectful to those groups of key natural enemies of the crop must be selected (Urbaneja et al., 2016), and the greatest number of insecticide modes of action should be alternated according to IRAC recommendations (Esteban Cortés, 2016).

Synthetic insecticides have been used to control pests on fruit trees after the discovery of DDT in the mid-1940s until the 1960s. In South Africa, several foliar insecticides like dimethoate, endosulfan, and the nematicide aldicarb have been registered and are used to control African Citrus Triozid (Le Roux et al. 2006). Yet even intensive insecticide programs against African Citrus Triozid (ACT) do not always prevent the spread of HLB, especially in new citrus plantings (Tiwari et al. 2011). However, insecticides applied on the stems and during the periods of profuse flush growths and offseason can greatly reduce ACT populations (Hall et al. 2012), and are recommended for ACT management (Fordjour Aidoo, 2019).

There are many studies that evaluate efficacy, side effects and resistance development with many active ingredients used for the control of D. citri. However, the number of studies that evaluate these parameters with T. erytreae are scarce and also old, so that the majority of new active ingredients have not been yet studied. In Spain, MAGRAMA authorized for the control of T. erytreae, a neonicotinoid with the trade name "Actara 25 WG", which contains 25% of the active ingredient thiamethoxam. The use of other active substances such as 20% Imidacloprid authorised for aphid and whitefly control have also shown good results. It is recommended to for to apply these insecticides during the citrus sprouting when environmental conditions are favorable for the development of the psyllid, i.e. low temperatures and high relative humidity (Esteban Cortés, 2016).

Until April 2019 (Regulation No. 2018/785 of May 29, 2018), the management of Trioza erytreae in the affected European territories was based mostly on chemical control by thiamethoxam. However, as of the aforementioned date, the use of thiamethoxam, imidacloprid and clothianidin has been absolutely prohibited and their extensions expired in outdoor crops. Acetamiprid is the only neonicotinoid whose use remains in force for European citrus today. Therefore, citrus safety in the Iberian Peninsula and the whole of European territory is currently without chemical control tools to manage this insect vector (Rizza, Hernández-Suárez, Perera, et al., 2019).

In this sense, there are other active substances under different chemical families of pesticides that are known to be effective against citrus psyllids. Dimethoate (organophosphate) would be especially interesting (Catling, 1969; Wortmann and Schafer, 1977; Tiwari et al., 2011; Qureshi et al., 2014).

Qureshi, Kostyk & Stansly (2014) evaluated the efficacy of different insecticidal treatments for the control of D. citri, using 49 products or 44 active ingredients (a.i), such as foliar spray or soil applications. These field trials were conducted in the State of Florida (USA), between 2005 and 2013, on sweet orange trees of Valencia (Citrus sinensis (L.) Osbeck (Rutaceae).) During this study a combined effect of suppression of nymphs and adults was obtained, with a reduction of more than 90% of adults of D. citri during the 24-68 days after treatment. The effect of foliar application (spraying) of insecticides tested on adults of D. Citri showed that 38 of the 42 products evaluated significantly suppressed adults (P, 0.05). An average reduction of 90 to 100% of adult psyllids was observed for 24 to 57 days (3 to 8 weeks), in comparison with untreated control, when were applied foliar sprays of tolfenpirad (Apta 15 SC), chlorantraniliprole

+ thiamethoxam (VoliamFlexi), lamda-cyhalotrine (Warrior II), metidation (Supracide 2 E), fenpropathrin (Danitol 2.4 EC), abamectin + thiamethoxam (Agri-Flex), dimethoate (Dimethoate 4 E), chlorpyrifos (Lorsban 4 E) and chlorpyrifos + zeta-cypermethrin (Stallion). The observable effects on nymphs were generally shorter; however, a reduction of 76 to 100% of nymphs was observed during 99 to 296 days post-treatment, in young trees treated with neonicotinoids: imidacloprid, thiamethoxam or clothianidin and a new anthranilic diamide, ciantraniliprol.

Qureshi, Kostyk & Stansly (2014) also evaluated the effectiveness of different insecticide treatments in combination with HMO (Horticultural Mineral Oils), products that we will refer to in the following section on biorational products. The results of these trials showed that application of some insecticides with HMO improved their performance against Asian citrus psyllid (ACP). Significant suppression of adults prolonged 7–14 days and improved 9–47% with the addition of an adjuvant to treatments of fenpropathrin (Danitol 2.4 EC), sulfoxaflor (Closer SC), spinetoram (Delegate WG) and diflubenzuron (Micromite 80 WGS). HMO by itself provided an average of 36% reduction in adults for 18 days and provided an average of 50% reduction in nymphs for 9 days.

The results obtained in a recent trial conducted by Rizza et al. (2019) in the Canary Islands, indicate that the selected formulations can be distributed in four efficacy groups for the chemical control of nymphs of T. erytreae. First, they highlight the rapid shock action of broad-spectrum pesticides with systemic activity, particularly organophosphate dimethoate (efficacy greater than 80% at 3, 7, 14 and 21 ddt); in a second efficacy group Lambda Cihalotrin, a pyrethroid that acts by contact and ingestion and shows some residual and repellent activity (showing an intermediate efficacy in the short-medium term, at all times greater than 60%); in a third group, spirotetramat and abamectin are embedded, which showed relatively low efficacy at the earliest trial dates (less than 50%) and high long-term efficacy (greater than 80%); and in a final efficacy group there would be exclusively pimetrozin, a modulator of cordotonal organs with systemic activity and acting by ingestion and contact (efficacy results below 50% in both short and long term).

Table 1. Potential chemical control of psilids in citrus

Active ingredient (S.T.)*	Type of pesticide (Trade name)	RESULTS/FINDING Psyllid(s) that controls and Biological(s) stage(s)	Cultivation	CEE authorized in CITRUS (Insects it controls)	Reference(s)
Abamectin 1,8% EW (10)	**Tetramic acid derived insecticide - Avermectin** (Cal-Ex Avance EW, Dynamec)	***Trioza erytreae:*** It showed a relatively low efficacy in the first days of the trial (≤50%), and a high long-term efficacy (> 80%). At 28 ddt, a high percentage of nymph mortality (91.19%) were obtained.	**Citrus**	**YES** (Mites and citrus miner)	Rizza et al. (2019) Burdyn, Hochmaier & Bouvet (2019)
Abamectin 1,8% EC (10)	**Tetramic acid derived insecticide - Avermectin** (Bermectine, Cal-Ex,				

	Dauparex, Marisol, Romectin, Safran, Apache, Vertimec, Vamectin 1.8 EC, Spidermec, Pickill, Laotta)	***Diaphorina citri:*** Control of nymphs			
Abamectin Agri-mek + Sol-Oil 97 (*0.15 EC FC435-66-10 oz/7 gal)(HMO)*	**Tetramic acid derived insecticide – Avermectin** (AGRI-MEK® SC) (Agri-mek + HMO)* (Agri-Flex)	***Diaphorina citri:*** Provided excellent knockdown of ACP adults 4-5 days after treatment (DAT) and good egg and nymphal suppression through 14 DAT. ***Diaphorina citri:*** According Qureshi, Kostyk & Stansly (2014), abamectin + thiamethoxam (Agri-Flex), is among the insecticide products of foliar application, where an average reduction of 90 to 100% of adult psyllids was observed, for 24 to 57 days (3 to 8 weeks), compared to the untreated control.	**Citrus**		Childers & Rogers (2005) Qureshi, Kostyk & Stansly (2014)
Acetamiprid 70WP (30)	**Neonicotinoide Insecticide** (Assail)	***Diaphorina citri:*** nymphs As indicated US-EPA (2015), begin applications as pest populations begin to appear. Thorough coverage is necessary for optimum control. The addition of a spray adjuvant such as silicone-based surfactants or horticultural oil may enhance coverage and improve pest control. Scout groves regularly and retreat if needed. Use higher rates under heavy insect pressure.	**Citrus**, Calamondin, Citron, Citrus Hybrids, Grapefruit, Japanese summer grapefruit, Kumquat, Lemon, Lime (including Australian desert, Australian finger, Australian round, Brown River finger, Mount White, Russell River, sweet, Tahiti, New Guinea Wild), Mandarin (Mediterranean, Satsuma), Orange(sweet, sour,	**YES** (white, gray and red louse; miner, Aphids and whitefly)	Childers & Rogers (2005) US-EPA (2015) Boina & Bloomquist (2015)

			tachibana, trifoliate) Pummelo, Tangelo, Tangor, Uniq fruit & Cultivars, varieties and/or hybrids of these.		
Acetamiprid 20% SG (14)	**Neonicotinoide Insecticide** (Gazel Plus SG, Epik 20 SG)	Control of ***Trioza erytreae*** According Dionisio et al. (2019), under field conditions, a percentage of 91.5 ± 1.6 was obtained, mortality of T. erytreae per shoot, at 28 days of treatment.	**Citrus**		Boina & Bloomquist (2015) Dionisio, Hernández-Suárez & Rizza, et al. (2019) (Jornadas Técnicas)
Acetamiprid 20% SL (30)	**Neonicotinoide Insecticide** (Carnadine)				
Acetamiprid 20% SP (14)	**Neonicotinoide Insecticide** (Mospilan, Epik, Cornalina, Acemur)				
Aldicarb (*15 G- 2 oz/tree)	**Systemic insecticide Nematicide** (Temik) Aldicarb [2-methyl-2-(methylthio) propionaldehyde *O*-methylcarbamoyl-oxime] is a systemic carbamate insecticide	***Diaphorina citri:*** Provided the longest residual control of nymphs through 19 DAT when the test was terminated. Very effective control of ACP. According Qureshi & Stansly (2008), aldicarb application at 5.6kg ha^{-1} to the bed side of mature citrus trees 2–3months before spring growth can suppress ACP through spring without a direct effect on principal psyllid natural enemies.	**Citrus**	**NO**	Childers & Rogers (2005) Qureshi & Stansly (2008)
		According to Fordjour (2019) aldicarb has been registered and are used to control ACT (***T. erytreae***) in South Africa.	**Citrus**	**NO**	Le Roux et al. (2006) cited in Fordjour Aidoo (2019)
Chlorpyrifos (*4 EC- 4 pints)	**Foliar insecticide** (Lorsban 4E- USA and Brazil)	***Diaphorina citri:*** Provided excellent knockdown of ACP adults 4-5 days after treatment (DAT) and	**Citrus**	**NO**	Childers & Rogers (2005) Qureshi, Kostyk &

		provided good suppression of ACP adults and nymphs through 18 DAT (good knockdown and residual activity against the Asian citrus psyllid). According Qureshi, Kostyk & Stansly (2014), chlorpyrifos (Lorsban 4 E) y chlorpyrifos + zeta-cypermethrin (Stallion), are among the insecticides of foliar application, that achieve adult psyllid reductions between 90-100%, for 24 to 57 days (3 to 8 weeks). Stansly & Qureshi, et al. (2019) recommend Lorsban 4E has a 2(ee) label for control of Asian citrus psyllid; because other formulations of chlorpyrifos are not currently labeled for psyllid control. Highly toxic to bees, do not apply during bloom.			Stansly (2014) Boina & Bloomquist (2015) Stansly & Qureshi, et al. (2019)
Dimethoate 40% EC (NA)	**Organophosphorus Insecticide** (Dafene Progress, Sistematon 40 Progress, Rubitox, Rogor L 40, Dimidon)	***Trioza erythreae:*** According to Rizza et al. (2019) is the best option to chemically combat the foci of *T erytreae*. It demonstrated an efficiency greater than 80% for the control of nymphs (at 3, 7, 14 and 21 ddt), effectiveness that increased towards the end of the test until reaching almost 100% at 28 ddt, being therefore effective both Short as long term.	**Citrus**	**YES** (mealybug, thrips and aphids)	Qureshi, Kostyk & Stansly (2014) Boina & Bloomquist (2015) Rizza et al. (2019) Fordjour Aidoo (2019) **https://www.phytoma.com/sanidad-**
Dimethoate 40% EC (120)	**Organophosphorus Insecticide** (Afithion, Danadim Progress, Rodime-40, Perfekthion Top)		**Citrus**		

		According to Fordjour Aidoo (2019) this insecticide has been registered and are used to control ACT (***T. erythreae***) in South Africa. Applied on the stems and during the periods of profuse flush growths and offseason can greatly reduce ACT populations. It is recommended only for seedlings without fruit production. Highly toxic to bees, do not apply during bloom. Stansly & Qureshi (2019) recommend not to make more than 2 applications per crop season. ***Diaphorina citri:*** According Qureshi, Kostyk & Stansly (2014), this product is among the insecticides of foliar application, that achieve adult psyllid reductions between 90-100%, for 24 to 57 days (3 to 8 weeks).			vegetal/avisos-de-plagas/psila-africana-de-los-citricos-trioza-erythreae Stansly, Qureshi, Stelinski, and Rogers (2019)
Dimethoate	**Organophosphorus Insecticide**	***Diaphorina citri:*** Effect on the population reduction of citrus psylla. Provided the almost completely control of the 7 days of application.	**Citrus**	**SI**	Sohail, Nisar and Rashad (2004)
Endosulfan	**Foliar Insecticide** (Thiodan 35 EC-Pakistan)	According to Fordjour Aidoo (2019) this insecticide has been registered and	**Citrus**	**NO**	Boina & Bloomquist (2015) Le Roux et al. (2006) cited

		are used to control ACT (***T. erythreae***) in South Africa. Applied on the stems and during the periods of profuse flush growths and offseason can greatly reduce ACT populations.			in Fordjour Aidoo (2019)
Fenpropathrin (*2.4 EC -16 oz)	**Foliar insecticide** (Danitol) (contains too petroleum distillates) (Danitol 2.4 EC),	***Diaphorina citri:*** Provided excellent knockdown of ACP adults 4-5 days after treatment (DAT) and gave good suppression of eggs and nymphs through 15 DAT. Restricted use pesticide. Highly toxic to bees, do not apply during bloom. ***Diaphorina citri:*** According Qureshi, Kostyk & Stansly (2014), this product is among the insecticides of foliar application, that achieve adult psyllid reductions between 90-100%, for 24 to 57 days (3 to 8 weeks).	**Citrus**	**NO**	Childers & Rogers (2005) Qureshi, Kostyk & Stansly (2014) Stansly, Qureshi, Stelinski, and Rogers (2019)
Imidacloprid 20% (14)	**Neonicotinoide Systemic Insecticide** (NOLEP 20 SL, KOPY, Couraze)	***Trioza erytreae:*** It is recommended not to treat in plots with the presence of grooved mealybug. Very dangerous for bees. To protect bees and other pollinating insects, do not apply during flowering of crops. Do not use where there are bees in active pecoreo.	**Citrus**	**NO**	https://www.phytoma.com/sanidad-vegetal/avisos-de-plagas/psila-africana-de-los-citricos-trioza-erythreae
Imidacloprid (*1.6 F- 10 oz)	**Foliar insecticide** (Provado)	***Diaphorina citri:*** Control adults and nymphs According Sohail, Nisar and Rashad	**Citrus**	**NO**	Sohail, Nisar and Rashad (2004) Childers & Rogers (2005)
Imidacloprid (*2 F- 0.25 oz/tree)	**Systemic Insecticide Neonicotinoide** (Admire)				

		(2004 have effect on the population reduction of citrus psylla. Provided the almost completely control of the 7 days of application. ***Diaphorina citri:*** According Childers & Rogers (2005): provided excellent knockdown of ACP adults 4-5 days after treatment (DAT). Good suppression of ACP adults and nymphs through 18 DAT, remained effective in suppressing nymphs through 25 DAT. Provided good residual activity against the Asian citrus psyllid. Provided very effective control of ACP, had significantly lower adult numbers 5 DAT. ***Diaphorina citri:*** According Qureshi, Kostyk & Stansly (2014), a reduction of 76–100% in nymphs or adults over 99–296 days was seen on young trees receiving drenches of the neonicotinoids imidacloprid (too thiamethoxam or clothianidin). Stansly and Qureshi, et al. (2019) recommend the limit of 0.5 lb AI/A per growing season regardless of application type (soil and/or foliar) and trade name of imidacloprid product used.			Qureshi, Kostyk & Stansly (2014) Boina & Bloomquist (2015) Esteban Cortés (2016) Burdyn, Hochmaier & Bouvet (2019) Stansly, Qureshi, Stelinski, and Rogers (2019)

Imidacloprid 20% SL (3)	**Systemic Insecticide Neonicotinoide** (CONFIDOR)	***Trioza erytreae:*** Positive results in semi-season conditions. High percentage of mortality of *T. erytreae* per shoot (7, 14, 21, and 28 days after treatment).	**Citrus**	**NO**	Dionisio, Hernández-Suárez & Rizza, et al. (2019) (Jornadas Técnicas)
Lambda cihalotrin (7)	**Insecticida Piretroide** (Warrior II)	***Diaphorina citri:*** According Qureshi, Kostyk & Stansly (2014), this product is among the insecticides of foliar application, that achieve adult psyllid reductions between 90-100%, for 24 to 57 days (3 to 8 weeks).	**Citrus**	**YES**	Qureshi, Kostyk & Stansly (2014)
Lambda cihalotrin 5%EG (7)	**Insecticida Piretroide** (Kaiso Sorbie)	***Trioza erytreae:*** According to Rizza et al. (2019) This pesticide seems to be slower, showing intermediate efficacy in the short-medium term (> 60%), which increases considerably as time passes (controlling efficacy close to 90%). A high percentage of nymph mortality was obtained: At 3 and 7 ddt (65.58%) and at 28 ddt (93.63%), and an average efficacy of 84.62%. Efficient in controlling ***D. citri*** (>80%) according to studies of Della Vechia et al. (2019)	**Orange tree Mandarin**	**YES** (fruit fly, mealybug, lice, aphids, whiteflies and lemon moth)	Rizza et al. (2019) Della Vechia et al. (2019)
Lambda-cihalotrin 10%CS (7)	**Insecticida Piretroide** (Pointer 100 CS, Karate Zeon, Karate Tecnología Zeon 10 CS, Arsinoe)		**Citrus**		
Lambda-cihalotrin 2,5% WG (7)	**Insecticida Piretroide** (Akira, Lamdex Extra, Lamdex Extra N, Lamdex 2,5WG)		**Orange tree**		
Methamidophos	**Organophosphate insecticide.**	***Diaphorina citri:*** Effect on the population reduction of citrus psylla.	**Citrus**	**NO**	Sohail, Nisar and Rashad (2004)
Metidation	**Organophosphate insecticide** (Supracide 2 E)	***Diaphorina citri:*** According Qureshi, Kostyk & Stansly (2014), this product is among the insecticides of foliar	**Citrus**	**NO**	Qureshi, Kostyk & Stansly (2014)

		application, that achieve adult psyllid reductions between 90-100%, for 24 to 57 days (3 to 8 weeks).			
Pimetrozin 25% WP (21)	**Cordotonal organ modulator insecticide** (Pulfly)	***Trioza erythreae:*** Rizza et al. (2019) obtained a low efficacy for nymph control (45.33%). Efficacy less than 50%, both short and long term. Only one treatment is recommended. It can be applied through drip irrigation by performing a single treatment per campaign at the beginning of the attack, using 800 g/Ha.	**Citrus**	**SI** (aphids)	Rizza et al. (2019) https://www.phytoma.com/sanidad-vegetal/avisos-de-plagas/psila-africana-de-los-citricos-trioza-erythreae Stansly, Qureshi, Stelinski, and Rogers (2019)
Pimetrozin 50% WG (21)	**Cordotonal organ modulator insecticide** (Plenum)		**Orange tree Mandarin Lemon tree**		
Spirotetramat 10% SC (14)	**Tetramic acid derived insecticide –** (Movento Gold)	***Trioza erythreae:*** Rizza et al. (2019) obtained a percentage of effectiveness in the control of nymphs of 79.51%. ***Diaphorina citri:*** Stansly & Qureshi, et al. (2019) recommend only to control psyllid nymphs, not adults. Limit of 0.32 lb ai per acre per 12 month. Minimum interval of 21 days between applications. During bloom, Stansly & Qureshi, et al. (2019), recommend only to control psyllid nymphs, not adults. Limit of 0.32 lb ai per acre per season. Do not make more than one application during the major citrus bloom period. They Also recommend to be applied in 2% horticultural mineral oil 97.	**Citrus**	**YES** (thrips, aphids, whiteflies, mites, mealybugs, lice)	
Spirotetramat 15% OD (14)	**Tetramic acid derived insecticide –** (Movento 150 O-Teq)		**Orange tree Mandarin Lemon tree**		

Tiametoxan 25% W.G	**Neonicotinoide insecticide** (ACTARA 25 WG)	***Diaphorina citri:*** Control adults and nymphs ***Diaphorina citri:*** According Qureshi, Kostyk & Stansly (2014), reduction of 76–100% nymphs or adults over 99–296 days was seen on young trees receiving drenches of the neonicotinoid thiamethoxam. Clorantraniliprol + thiametoxam (VoliamFlexi) is also among the insecticides of foliar application, that achieve adult psyllid reductions between 90-100%, for 24 to 57 days (3 to 8 weeks). Efficient in controlling ***D. citri*** (>80%) according to studies of Della Vechia et al. (2019) Stansly & Qureshi et al. (2019) recommend not to exceed a total of 11.0 oz/A (0.172 lb AI/A) of Actara or 0.172 lb a.i. of thiamethoxam-containing products per acre per growing season. Do not apply during pre-bloom or during bloom when bees are actively foraging. ***Trioza erythreae:*** It is recommended to carry out a treatment without exceeding 600 g/ha, can also be treated by drip irrigation in a single application at a rate of 0.6-0.8 g/tree (authorized for aphids and whiteflies)	**Citrus**	**NO**	Qureshi, Kostyk & Stansly (2014) Boina & Bloomquist (2015) Burdyn, Hochmaier & Bouvet (2019) Della Vechia et al. (2019) https://www.phytoma.com/sanidad-vegetal/avisos-de-plagas/psila-africana-de-los-citricos-trioza-erythreae Stansly, Qureshi, Stelinski, and Rogers (2019)

Tolfenpyrad	**Belongs to the Mitochondrial Complex I Electron Transport Inhibitors** (METI 1), which work by inhibiting cellular respiration in the mitochondria (Apta 15 SC)	***Diaphorina citri:*** According Qureshi, Kostyk & Stansly (2014), this product is among the insecticides of foliar application, that achieve adult psyllid reductions between 90-100%, for 24 to 57 days (3 to 8 weeks).	**Citrus**	-	Qureshi, Kostyk & Stansly (2014)
Use of broad spectrum insecticides	**Rotate modes of action (pyrethroids, OPs Carbamates)** Chose from recommended products based on PHI and previous history	A dormant spray is a foliar application of broad-spectrum insecticide directed at overwintering adult psyllids. Dormant Season (November to February): Pyrethroids Carbamates (short PHIs) Soil applications. Post-bloom (Marche-May): selective insecticides if needed: Imidacloprid drench young trees. Summer (Jun - october): Organophosphates, carbamates or selective insecticides if needed.	**Mature Citrus**	**NO**	Stansly (2010)
Management using a broad spectrum insecticides	Due to restrictions on the amount of neonicotinoid insec-ticide products that can be used per growing season, the number of allowed applications in solid plantings of trees 5–9' in height is greatly limited. It is also important to note that imidacloprid, thiamethoxam and clothianidin are not considered alternatives for rotation to prevent resistance because these insecticides share the same	Foliar insecticide applications to mature trees during the growing season are best made with selective insecticides to minimize impact on natural enemies that help control psyllids and other pests. Management practices used within a grove can affect psyllid populations, especially those practices that promote new flush such as hedging, topping, and fertilization. Trees should always be	**Citrus**	**NO**	Stansly, Qureshi, Stelinski, and Rogers (2019)

	mode of action. Foliar sprays of products with modes of action other than the ones used in drenches should be used between soil-drench applications to provide additional control of ACP and to help minimize pest selection for insecticide resistance development.	sprayed with a broad spectrum insecticide prior to or just after hedging and topping and before flush develops. Management strategies that reduce or limit the duration of flush may help to keep psyllid populations at low levels and reduce the need for additional pesticide applications.			

*The safety term (S.T.) is expressed in days or not applicable (NA)
*Horticultural mineral oil (=HMO)

3.2.2. Phytosanitary Control in Ecological Citriculture

3.2.2.1. Introduction and Legislation

The need to produce in a way that avoids and controls the progressive environmental deterioration, has been changing the concept of agriculture towards sustainable agriculture that tries to exploit natural resources without compromising the development of future generations, so that not only act from an economic point of view but also ethical and environmental; with this in mind, Integrated Production arises. The International Organization of Biological Control (IOBC) defines it as a system of agricultural exploitation that produces food and other products of high quality, using natural resources and regulatory mechanisms, to replace polluting inputs and to ensure sustainable agricultural production.

Recently, in 1991, the European Directive 91/414/EEC of the Council, of 15 July, concerning the marketing of phytosanitary products, defined the integrated fight (protection) as *“the rational application of a continuation of biological measures, biotechnological, chemical, crop, or vegetable selection, so that the use of chemical phytosanitary products is limited to the minimum necessary to keep the population of the pest at levels below those that would cause damage or losses unacceptable from the point of view economic"*. In each of these definitions a common basis is observed: all rely on chemical control, but seek to relegate it to the last resource, giving priority to natural methods and those techniques that are more environmentally friendly; it also based on the knowledge of the harmful agents that are intended to control, to specify the optimal time to perform the intervention in case this is necessary, according to the level of plague that exists and the state of the crop, valuing the action of the natural enemies and other regulatory factors.

In terms of Ecological Production, in order to adapt to this new scenario, a series of agricultural methods that approximate agronomy to ecology have been put into operation, which together are known as «Sustainable or Sustainable Agriculture». This denomination of «sustainable agriculture» is the last link in a conceptual chain that turns around the practice of an alternative to intensive agriculture, which has been accompanied by a wide spectrum of different adjectives over time (ecological, biological, organic, biodynamic, etc.).

The ecological agriculture has been legally regulated in Spain since 1989, when the Regulation of the Generic Designation " Ecological Agriculture " was approved, which was applicable until the entry into force of Regulation (EEC) 2092/91 on organic agricultural production and its indication in agricultural and food products. Currently, since January 1, 2009, the date on which it has entered into application, organic production is regulated by Regulation (EC) 834/2007, the Council on production and labeling of organic products and by which it is repealed in Regulation (EEC) 2092/91 and by the Regulations: R(CE) 889/2008 of the Commission, laying down provisions for the application of R (EC) 834/2007 with respect to organic production, its labeling and Control and R(CE) 1235/2008 of the Commission establishing the implementing provisions of R(EC) 834/2007, as regards imports of organic products from third countries.

Regulation (EC) 834/2007, and all its provisions and modifications, establishes, among other precepts: the obligation to subject farmers, importers and processors, who wish to market Ecological Production products, to a control regime to ensure that production standards are respected, and that techniques incompatible with this agricultural system of food management and production are not used.

3.2.2.2. Crop Health in in Ecological Citriculture

The main pests and diseases that affect the organic cultivation of citrus fruits are the same as those found in conventional cultivation, but, given that there are fewer means of direct control, it is essential to maintain the crop in a satisfactory state through all preventive measures. It is necessary to understand the crop as a whole and consider the development of a disease or the proliferation of a pest as the expression of an imbalance before which we must adopt a set of measures or changes in order to restore balance.

In order to guarantee the health of the crop under organic production conditions, it is essential to maintain the highest possible biological diversity, since the regulations of potentially harmful organisms are higher and more stable. The work and fertilization must be seen within a global approach to plantation health. The use of phytosanitary products, even with authorized natural substances, should not be the first option for phytosanitary control, it should be based on models that prioritize the management of biodiversity (such as cover plant and more suitable hedges or associated auxiliary fauna), Good agricultural practices in the cultural control of cultivation and Biological Struggle.

Among the ecological practices recommended to maintain citrus health are the fallowing (Porcuna Coto, Gaude Soriano, Castejón de Romeros & Dominguez-Gento, 2010):

- Regular contributions of organic matter, green manures, fodder crops, pruning remains, etc.
- Diversity of crops: Permanent vegetal cover as long as possible (keep it at least until after the first sprouting), hedges alive around or between the crop (on edges and fringes or slopes of interior terraces, forced on borders with integrated production crops as neighbors).
- Reduced soil work with light tools.
- Enough and moderate irrigation. Adequate drainage. Citrus cultivation suffers more from excess than from lack of water. Aeration of the neck of the trees preventing water from suffocating the roots.
- Prunings little energetic and annual, adapted to each variety. Aerate without opening excessively.
- **Moderate or no use of natural biocides. It is preferable to use natural reinforcing or repellent preparations.**

In ecocitriculture, biodiversity is a basic pillar for the maintenance of fertility and crop health. **The weed flora and the adventitious plants together with the green manures or vegetal coverings** (cultivated or of the weeds themselves) perform beneficial functions such as, stimulate the biological activity of the soil, improve its structure, protect from erosion, decrease the leaching of the nutrients, etc.

I. Arthropod management

An interesting association that we must make in organic citrus cultivation **are the hedges**; these serve as a natural border, isolating from environmental pollutants (sometimes too close and numerous). **They will also serve as refuge and alternative food supply for auxiliary fauna**, such as herbaceous plants, for example: the control of aphids and other phytophagous is closely related to these wild species.

In this way, good management of biological diversity together with good agricultural practices would lead to stability of the agroecosystem equilibrium. According to Porcuna Coto, et al. (2010), in organic citrus the organisms that most often cause economic problems are usually hemiptera; and attention must be focused primarily on three diaspids: the California red scale, the gray scale and purple scale. The rest of arthropods (mealybugs, aphids, mites, whiteflies, leafminer) will only cause discomfort in specific situations (stress, sensitive varieties, early age, etc.), since in ecological conditions these phytophagous are generally well controlled by their predators and parasites (Porcuna Coto et al., 2010).

The case of mealybugs or coccids also has importance in ecocitriculture. Since, in general, the tree continues to grow well, the damage that obliges the farmer to carry out a treatment is the commercial depreciation of oranges, due to the discolorations that occur in fruits, as well as their direct presence on the fruit skin (Porcuna Coto et al., 2010).

The actions that are proposed to control the populations of **the California red scale (*Aonidiella aurantii*** (Maskell)) are, on the one hand, to create niches suitable for predators and parasites, such as hedges adjacent to copious and winter blooms, that attract to others other mealybugs, which serve as an alternative food to parasitic wasps of the genus *Aphytis.* Despite having higher percentages of parasitism in organic gardens, **so far it is not possible to fully control the scale naturally. Therefore, it is recommended to carry out treatments with mineral or paraffinic oils, 1-2%** (Porcuna Coto et al., 2010).

Currently among the products of plant origin, a natural Insect growth regulator (IGR) is used, whose active ingredient is extracted from the seeds of the fruits of a plant (*Azadirachta indica* A. Juss). In Spain, ***A. melinus*** is the top competitor and has displaced the native parasitoid ***A. chrysomphali*** in the southern areas of the Iberian Peninsula, since it can better tolerate high temperatures in summer, however, in the northwest both coexist from the introduction of the first, and it has been suggested that this occurs because neither of them has been able to control populations of ***A. aurantii*** (Sorribas, 2011; Pekas, 2011; Cebolla, 2018).

Both ***A. melinus*** and ***A. chrysomphali*** accept ***A. aurantii*** equally and at high proportions, which has implications for augmentative biological control (ABC) programs, in which ***A. melinus*** releases are being recommended; to improve the release it would be recommended that the parasitoids had access to the hosts before the releases, so that they tended to parasitize when they are released in the field instead of using the hosts as a power source (Bru Martínez, 2014).

In Spain, several methods are used to control the populations of *A. aurantii* that range from cultural ones such as pruning, to chemical, biological and biotechnological ones that include sexual confusion (Bru Martínez, 2014). This technique recently developed in our country (Vacas et al., 2009; Vacas et al., 2010; Vacas et al., 2012) does not leave residues in fruit, does not pose any risk to human health and is compatible with others methods such as biological control (Vacas, Alfaro, Primo, et al., 2015).

The technique of sexual confusion of the California red scale as a control strategy has been developed at the Polytechnic University of Valencia as a method of biorational control of this pest; **Its application is simple and consists of placing, once a year, between 400 and 500 steam diffusers per hectare that release pheromone in a controlled way to the environment.** With this arrangement and this type of emitters it **is achieved that a sufficient pheromone concentration is achieved in the environment to interrupt the chemical communication between males and females and, in conclusion, prevent the males of the species from finding the receptive females. This prevents this plague from reproducing and, therefore, during the successive generations the populations of this insect are reduced** (Vacas et al., 2009; Vacas et al., 2010; Vacas et al., 2012; Vacas, Alfaro & Primo, et al., 2015). **In addition,**

the combination of this technique of technological control, with the application of mineral oils has resulted in levels of efficiency above 90%. The application of the technique causes a delay in the development of the pest that favors the action of parasitoiodes by prolonging the period of susceptibility of the insect (Germán Casado Mármol et al., 2016).

When populations of the California red scale escape control of the parasitoid, the only biotic factors capable of maintaining the development of *A. aurantii* are predators. These natural enemies are effective in regulating high densities of ***A. aurantii*** and although they are not very specific, they have been used through inoculative and augmentative releases (Siscaro et al., 1999). In Spain, three species of coccinellids have been cited on diaspids (coccidea), which are: *Rhyzobius lophantae* Blaisdell, *Chilocorus bipustulatus* (L.) and *Exochomus quadripustulatus* (L.) (Moner, 2000; Urbaneja et al., 2005; Sorribas and Garcia-Marí, 2010; Bru Martínez, 2014)

In other citrus regions, some neuropteran from the *Chrysopidae* family and the ***Conwentzia psociformis* (Curtis)** (Neuroptera: Coniopterygidae) have been observed attacking various ***A. aurantii*** states for food. The thrips ***Aleurodothrips fasciapennis*** (Franklin) and **mites of the *Hemisarcoptidae* family** have also been cited. In Spain, some cecidomids dipterans have been observed depredating ***A. aurantii*** (Vanaclocha et al., 2012), as in the case of the species ***Lestodiplosis aonidiellae*** (Sorribas, 2011), also the **phytoseid mites *Typhlodromus phialatus*** (Athias-Henriot) and ***Amblyseius swirskii*** (Athias-Henriot) (Juan Blasco et al., 2008; Bru Martínez, 2014).

Very recent studies have shown that predation is the main biotic component of mortality from ***A. aurantii***, causing reductions of more than 75% in recently settled cohorts. Bouvet et al. (2019) detected ***Aonidiella aurantii*** DNA in the digestive system of 11 species of predators, among which stand out for their higher proportion of positive detection: ***Pilophorus cf gallicus, Rhyzobius lophanthae, Semidalis aleyrodiformis, Scymnus interruptus, Cheiracanthium mildei, Scymnus subvillosus*** and ***Chrysoperla carnea.*** In this case, generalist and stenophagous predators, mainly associated with other citrus pests such as aphids, proved to be the most important biological control agents of this pest (Bouvet, Urbaneja, Pérez-Hedo & Monzó, 2019).

For the rest of the diaspids, especially gray scale (*Parlatoria pergandii*) and purple scale (*Lepidosaphes* spp.), the same approach is used, with the exception of observing the biological cycle of each insect. It must be taken into account that on many occasions, by concentrating the efforts to control *Aonidiella*, we run the risk of firing the populations of *Parlatoria*, given that treatments to reduce sensitive individuals are not being adequately carried out (Porcuna Coto et al., 2010). According to experiences in this regard (Domínguez Gento 2007), with correctly performed treatments, more than 70% efficiency can be achieved (which means of the order of 90% of the orange of 1st commercial category, with less than 5% of depreciation for these circumstances).

Other treatments with sodium silicate (3-5%) or calcium polysulphide (1-5%) seem to offer good results in other world ecocitrus areas. Vegetable oils appear as a possibility of substitution of mineral oil, but there are not yet enough studies to support their efficacy on citric acids. In some doses or conditions, the polysulfide could become phytotoxic (Domínguez, Chulià and Bolinches, 2003), as well as some formulations of vegetable oils, so we must be very careful (Porcuna Coto et al., 2010).

In some special case, such as **cotonet (*Planococcus citri*), biological control can be applied as a direct measure, releasing *Cryptolaemus montrouzieri* (from 5 to 10 individuals per affected tree) and *Leptomastix dactilopii* (10 to 20 / tree). Other practices to prevent its proliferation** would be to keep the tree well trimmed so that it is aerated, in a wide plantation frame or to **carry out treatments with 3% potassium soap, cleaning the insect exudate** (Porcuna Coto et al., 2010).

As for aphids, mainly **Cotton aphid (*Aphis gossypii*) and Spirea aphid (*Aphis spiraecola*),** the main damage caused by these insects is the weakening of the tree due to sap suction; Since ecological plantations do not usually have sprouts too tender (not having abundant or excessive nitrogen) both these, as the rest of sucking insects, are not usually excessively problematic organisms. In this type of ecological management, it is essential to have a minimum of patience. They usually end up being preyed upon or parasitized by parasitic hymenoptera, Coccinellid beetles (ladybugs), neuroptera (crisopa and conwentzia), Syrphid or Cecidomid dipterans, that are the most abundant predators in ecological crops; fungi of the genus *Verticillium*, complement the action of the previous ones. In the control of this pest the maintenance and improvement of the vegetation cover and other habitats for reserves of useful fauna plays a fundamental role, the graminean covers can significantly improve the effectiveness of the biological control that mainly *Scymnus* and cecidomids exert.

The abundance and diversity of predators does not ensure efficient biological control for ***A. spiraecola*** in citrus fruits, because there may be asynchrony between the population peaks of the pest and of the predators (predator-predatory dynamics), which is usually a limiting factor of their efficacy in all agroecosystems with explosive pests such as aphids (Welch and Harwood 2014). A study by Gómez-Marco (2016) **showed the positive effect that an early arrival of predators would have on the colonies of *A. spiraecola*, concluding that the presence of predators at the beginning of the aphid season should be considered for the development of new treatment thresholds and for biological control programs. These authors evaluated the management of plant coverings planted with grasses as a biological conservation control strategy. These plant species harbored stenophagous aphids of grasses such as *Rhopalosiphum padi* L. and *Sitobion fragariae* Walker (Hemiptera; Aphididae), which appeared two months earlier in this ecosystem with respect to citrus aphids, serving as alternative prey for predators increased their number by start of the season Consequently, citrus plots with vegetation cover tended not to exceed the treatment threshold** (Gómez-Marco, 2016; Gómez-Marco et al., 2016).

Several factors can affect the biological control by parasitoids of *A. spiraecola* in citrus fruits. One of them may be the presence of hyperparasitoids in this agroecosystem (Gómez-Marco et al. 2015), because hyperparasitoids are hymenopterans that lay eggs in an aphid mummy (i.e. an already parasitized aphid), and within this, they kill the larva of the primary parasitoid, emerging from the mummy an adult hyperparasitoid. In a study by Gómez-Marco (2016), they confirmed that the parasitism rates of ***A. spiraecola*** were low (less than 5%) and identified the species *Binodoxys angelicae* Haliday (Hymenoptera: Braconidae) as the only primary parasitoid that emerged of the mummies of A. spiraecola (Gomez-Marco et al., 2016).

Bouvet, Urbaneja & Monzó (2019) studied different effects on the population dynamics of aphids in the cultivation of citrus fruits (clementine mandarines**). These authors determined that the success of biological control was only achieved through the early presence of natural enemies, such as predatory kitchens of the genus *Scymnus* sp.**, with the ability to efficiently regulate aphid populations in the colonization phase. Therefore, their results suggest that conservation strategies, aimed at preserving and improving ***Scymnus* sp.** populations, are an important contribution to the future success of the biological control of ***Aphis* sp.** as a key plague of citrus fruits (Bouvet, Urbaneja & Monzó, 2019).

In some specific case, when biological control is not enough, we can act with treatments from 2-4% potassium soap, recommended if there is molasses, rock dust, with *Lithothamne* (crushed calcareous algae) or wood ash, which they strengthen the buds and dry the aphids. If the leaves are rolled we can use **neem or quasia extract, of sensitive systemic effect,** which can **be applied alone or in combination with paraffinic oil (1-1.5%). Preparations based on decoction of horsetail (*Equisetum arvense*) and nettle slurry (*Urtica dioica* L.), give strength (hardness and nutrients) to the plant**, while they can act as repellents. **Garlic extract can also have a repellent effect. Diatomaceous earth, kaolin or crushed minerals (silica or rock dust), can also harden sprouts against suckers or mites, while they can dry out and dehydrate many of these arthropods** (and in some cases protect against spring fungi, which can occur due to excess moisture) (Porcuna Coto et al., 2010).

Lepidopterans are usually already minor problems. We must promote larger predators, such as insectivorous birds. **For citrus flower moth (*Prays citri*) we can place traps with pheromones, for monitoring or massive captures and deal with *Bacillus thuringiensis* for the control of larvae** (Porcuna Coto et al., 2010).

In the case of the citrus leafminer (*Phyllocnistis citrella* Stainton) we should only intervene in case of seedlings or reinserted, since the presence of adults does not affect production at all. Experiences have been carried out with different natural products in clementine seedlings (Dominguez, Lanchazo, Armengol, Carot, 2003), **with 0.25% azadirachtin painted on the trunk is enough for its control.** Other effective permitted products **are garlic extract or *Bacillus thuringiensis*** (Porcuna Coto et al., 2010).

Something more problematic is **the Mediterranean fruit fly (*Ceratitis capitata* Wied).** Despite not being a widespread problem on all types of citrus fruits, their damage can be significant in early clementines (Marisol, Loretina, Beatriz, etc.) and in late varieties (Valencia-Late type). **Currently, an effective way to control it is the massive trapping, which consists of capturing adults using traps and different types of synthetic attractants, with the aim of capturing and eliminating a proportion of individuals high enough to reduce the hurt. Traditional traps are glass or plastic flycatchers, which may contain 1% hydrolyzed protein, ammonium phosphate or apple or vinegar juices (attracts females) and insecticide (approximately 70% attractant and 30% insecticide). The use of Tri-pack®, a female attractant, which can be used alone or in combination with an insecticide, formed by the mixture of three components (ammonium acetate, putrescine and trimethylamine), has also been widespread, being formulated more efficiency than the old ones based on liquid hydrolyzed proteins. A small dose of insecticide is placed next to the food attractant to kill the captured flies. Authorized insecticides are pyrethrins for use in traps or, neem or Spinosad** (Porcuna Coto et al., 2010; Monzó, 2010; Germán Casado Mármol et al., 2016).

Parallel to the massive trapping, there are other strategies for technological control of the Mediterranean fruit fly used today, chemosterilization and sterile insect technique or self-control. Chemosterilization seeks to reduce pest populations, by reducing the fertility or fertility of individuals in a population, by coming into contact with a chemical that negatively affects these parameters. Likewise, the autocidal or technical control of the sterile insect technique (SIT), is based on the sterilization by irradiation of a large number of males who, when released, compete with the wild males for fertilizing the wild females. The offspring produced by females that have copulated with these irradiated males is not viable, which has a negative impact on the wild populations of this pest (San Andrés et al., 2009). SIT programs are a non-polluting method of prevention, suppression or eradication of ***C. capitata*** that is being applied in different countries (Linquist 2000, Hendrichs et al. 2002; Monzó, 2010; Germán Casado Mármol et al., 2016).
Regarding the biological control of *Ceratitis capitata*, it has been based mainly on the augmentative releases of parasitoids. In recent years, more attention is being given to augmentative biological control, based primarily on the release of two species of braconids, *Diachasmimorpha tryoni*, larval parasitoid and *Fopius arisanus*, egg parasiotide (Monzó, 2010). Other potential agents of biological control of *C. capitata* are polyphagous predators present in the soil of this crop; taking into account that, the Mediterranean fruit fly develops part of its biological cycle in this environment, both third stage larvae, as well as pupae and newly emerged adults, are sensitive to the action of these predators. There are some works that cite ants, carabids, staphyloids and spiders, such as larval predators and fruit pupae (Eskafi and Kolbe 1990; Galli and Rampazo 1996; Urbaneja et al. 2006). On the other hand, Monzó (2010) in his study on this subject, found a great wealth and abundance (activity-density) of polyphagous predatory arthropods present in the soil of citrus agroecosystem in the Spanish Mediterranean: spiders with 51 species and 20 families they constituted the group of predators with greater diversity, being the *Pardosa sieve* lysoside the most abundant species; the group of staphyloids was the

second in species richness, being *Atheta mucronata*, new appointment in the Iberian Peninsula, the most abundant; likewise, the *Pseudoophonus rufipes* carabid represented more than 50% of the total of carabids and the *Forficula auricularia* dermaptero was the most abundant species of this group in all the plots.

It is necessary to consider a series of essential cultural measures that allow the control of the fruit fly, reducing the populations of this phytophagus. Among such measures are, the control of foci through the elimination of infested fruits, harvesting and subsequent burying of unharvested fruit, and the removal of isolated or abandoned fruit trees found within the citrus area. In this regard, an influence of the type of vegetation cover management on the abundance of predators has also been found, according to the study by Monzó (2010) in the plots where there was a spontaneous or cultivated vegetation cover, a greater abundance of predators.

It has also been introduced the use of insecticides of natural origin, such as Spinosad, a natural product obtained by the fermentation of the bacterium *Sacharopolyspora spinosa,* of the order of Actomycetales, the action of which is produced by the toxin metabolized by the bacteria (spinosyn), which has an insecticidal effect (similar to the toxins of *Bacillus thuringiensis*), included in the regulation of ecological agriculture. The treatment of patching on the south face with 0.20% Spinosad mixed with food attractant (hydrolyzed protein) is now the best (even more effective) alternative to mass trapping.

Other arthropods present in our organic citrus orchards are phytophagous mites, commonly known as "spiders”. **The most abundant in our territory are *Tetranychus urticae* (Red spider mite) and *Panonychus citri* (Citrus red mite).** The varieties most sensitive to these tetraniquids are those of the Clementines. **Fortunately, they are almost always accompanied by phytoseid mites (their most effective natural enemies, such as *Euseius stipulatus, Thryphlodromus phialatus, Amblyseius californicus*), cecidomids (very effective predatory flies) and some coleoptera coccinellid (*Chilocorus bipustulatus, Stethorus punctillum*) or chrysopids (*Conwetzia psociformis*, Semidalis, crisopas), which keep them within rational limits.** Only in sensitive varieties, such as clementines, has minimal damage been observed in dry seasons**. That is when you can perform some natural treatment with paraffinic oil (1-1.5%). Sulfur, being also effective, can affect phytosides precisely, leading to reinfestations in a few days; however, it can be used in its wettable form. The addition of neem or azadirachtin oil (its active ingredient with the greatest effect, 0.2%) to the mineral or vegetable oil, improves their effectiveness, giving satisfactory control.** In the case of the lemon tree, the citrus bud mite (*Aceria sheldoni* (Erwing)) can cause damage to the spring sprouts; action should be taken if 20% of affected shoots are present, before the spring move (shoot <5 cm); It can also be treated at the beginning of the summer sprouting (Porcuna Coto et al., 2010).

In Spanish citrus fruits 12 species of phytoseids have been identified (García-Marí et al. 1986; Abad-Moyano et al. 2009a), **at least 8 of these species have been described in the vegetal cover** (Aucejo-Romero, 2005; Aguilar -Fenollosa et al., 2011a). **The most important and commonly found species in the cups of clementine**

orchards associated with colonies of *T. urticae* and / or *Panonychus citri* (McGregor) (Acari: Tetranychidae) are: *Euseius stipulatus* (Athias-Henriot), *Phytoseiulus persimilis* Athias-Henriot, *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) and *Thyphlodromus phialatus* (Athias-Henriot) (Abad-Moyano et al., 2009a).

Regarding the biological control strategies of *T. urticae*, much emphasis has been given in recent years to biological control by conservation through vegetal cover management and biological control by inoculation through the augmentative releases of phytoseid mites. About biological control by conservation through the management of the vegetal cover, the use of the grass *Festuca arundinacea* Schreber (Poaceae) in the regulation of *T. urticae* populations has been demonstrated. The fescue sown between crop lines, acts as a reservoir keeping the populations of native or native natural enemies (mainly phytoseidos) elevated throughout the year (in addition if it is left to glean, pollen is used by phytoseids as food). In this way, predators act by regulating and decreasing the presence of ***T. urticae*** in trees (Aguilar-Fenollosa et al. 2011a, 2011b, 2011c; Aguilar-Fenollosa et al., 2012). Therefore, the use of this plant cover is being widely recommended to farmers.

In general, is important to note that, with a good management of the vegetal cover and hedges, it is found that these secondary arthropods can be controlled, such as aphids, whiteflies or mites (Garrido, 1999). Both in the case of *Ceratitis*, like in slugs and snails, the predatory fauna is of great importance to avoid the excessive population that can cause problems.

In the case of molluscs, it is vitally important to prevent them from climbing on the seedlings, especially in spring, since they will gnaw young shoots, leaves and even the trunk, reaching to paralyze their growth or dry a large part of the tree**. It should therefore be applied to the soil of iron sulphate or ferric triphosphate, as soon as its presence is detected in seedlings and grafted shoots; the branches should be shaken before treating, to eliminate those that are above,** repeating the treatment as many times as necessary if the product is moistened (and loses efficacy) or the problem reappears. You can also place traps with water, brewer's yeast and molluscicide, to remove them, but it is usually more expensive. Ducks or other domestic birds are also large snail predators.

The western flower thrips (*Frankliniella occidentalis*), is one of the most abundant in our citrus fruits according to a study by Navarro et al. (2008). This thrips and others also phytophagous, that could be found eating pollen or on fruits, do not cause remarkable damage; Even some species has a certain degree of interest in its behavior as a predator (***Aeolothrips* spp**). **As of 2007, serious damage caused by *Pezothrips kellyanus* is observed in the Valencian regions, which have been amplified and spread throughout the citrus territory** (Porcuna Coto et al., 2010). Recently an increase in damage caused by **orchid thrips (*Chaetanaphothrips orchidii)*** has been detected in the south of the province of Valencia. **If there is a risk, for the area in which the plot is located, of possible infestation of this thrips, it is recommended to perform treatments with oils (minerals or vegetables), together**

with some natural insecticide (Spinosad, neem, quasin, pyrethrin), to reduce spring populations (in the flowering-curd season). From the point of view of cultural control for the control of Trips, the presence of flowering plants before citrus fruits, on the plot or at their edges, should be avoided, because the thrips could reproduce in these plants, and therefore more abundant and harmful when citrus blossoms (García-Marí, 2012).

The most important biological control agents of *Pezothrips kellyanus* are the predatory mites present in the soil, so that a greater abundance of soil mites is linked with a smaller population of thrips; among them, the mite *Gaeolaelaps (Hypoaspis)* sp. (Acari: Laelapidae). The natural enemies of *P. kellyanus* present in the treetops are mostly general predators (myrids, chrysopids, coccinellids and other thrips) and do not seem to have a very important impact on the abundance of their populations. Although there are no specific cultural practices recommended, it is also known that the management of the soil vegetal cover can also influence the damage (García-Marí, 2012).

Finally, ants can also cause headaches for citrus growers, especially **the Argentine ant (*Linepithema humile* (Mayr))**, **which is the most abundant above the orange trees of our plantations. Ants have the double action, on the one hand they act as predators, especially of arthropods that at one time in their cycle can live on the ground, such as Ceratitis**, **and on the other, as an orange plague, at least indirectly, due to the defense and breeding of aphids they perform, driving away predators and parasites of the latter, and thus hindering their biological control.**
Calabuig et al. (2015) in their study "*Ants in citrus: impact on the abundance, species richness, diversity and community structure of predators and parasitoids",* carried out ant exclusion trials in three commercial citrus orchards, each dominated by an ant species (*Pheidole pallidula, Lasius grandis or Linepithema humile*) for two consecutive years, and compared the abundance, species richness, diversity and community structure of predators and parasitoids, between treatments with and without the presence of an ant. According to their results, these authors suggest that ants in citrus fruits are not associated with a dramatic decrease in the abundance or biodiversity of the natural enemy, on the contrary, ants were associated with greater richness and diversity of parasitoid species; however, on the other hand, ants negatively affected the abundance of specific natural enemy species, mainly general predators, and the impact on these predators could explain the higher pest densities associated with ants in citrus fruits (Calabuig et al., 2015).
As control measures, the aphids that support ants must be fought; It is also possible to associate repellent plants or carry out treatments with extracts thereof (mint, garlic), but they have no lasting or total effect. The sticky traps around the trunk may be initially interesting, but they also manage to bypass them by building "walkways" of their own individuals.

Table 2. Strategies and actions for the Ecological Phytosanitary Control of some important arthropod pests in citrus cultivation (Table modified and updated as published by Porcuna Coto et al., 2010)

Pest*	Follow-up methodology * Intervention Threshold *	Ecological alternatives*
California Red scale *(Aonidiella aurantii)*	Observation of fruits occupied in the previous year and population sampling. Chromotropic and sexual traps. Sampling during collection, observe 200 random fruits and determine a % of fruits attacked. **Thresholds:** If 2% or more of affected fruit is observed in the previous campaign it is recommended to treat to the maximum to sensitive forms in the first generation. - In pending harvest, if 2% or more of affected fruit is observed in the second generation, treat the sensitive forms (young nymphs) with oil.	**Maintenance and improvement of vegetal covers and other habitats for wildlife reserves.** **Loose entomophageal fauna:** - Unleash the predator *Cryptolaemus montrouzieri* in early spring to control coconet. - Perform massive release of the parasitoid *A. melinus* for the control of red and white scale. **Use of authorized organic products, biocides or natural preparations:** - Mineral oil (1-2%) - Paraffin oil (1.5-2%) - Potassium soap (1-3%) - Calcium polysulfide (1-3%) - Sodium silicate (0.3-5%) **Technological control technique:** The technique of sexual confusion as a method of biorational control of the California red scale in citrus fruits. This technique is based on the use of numerous emitters of a synthetic sex pheromone, similar to that of the insect, in order to saturate the culture environment and thus hinder the location of adult females by flying males. Thus, by using 400 pheromone diffusers per hectare, reductions in fruit damage of up to 70% can be achieved compared to control in early varieties, and 45-60% in late varieties. This prevents this pest from reproducing and, therefore, in the course of successive generations the populations of this insect are reduced. In addition, the combination of this technique of technological control, with the application of mineral oils has resulted in levels of efficiency greater than 90%. The application of the technique also causes a delay in the development of the pest that favors the action of parasitoiodes by prolonging the period of susceptibility of the insect (Germán Casado Mármol et al., 2016).
Oleander scale (Aspidiotus nerii)	Similar to California red scale	
Purple scale (Scale) (*Cornuaspis* sp.= *Lepidosaphes beckii* Newman, *Insulaspis gloverii*)	Observation of fruits occupied in the previous year and population sampling. **Threshold:** More than 2% of the fruit affected in previous harvest, treat first generation to the maximum of sensitive forms. If it is less than 2% tartare in second generation if necessary.	
Cotonet *(Planococcus citri)*	Observation of presence in fruits. **Threshold:** Treat only if more than 20% of the fruits are infested with cotonet.	
Mediterranean black scale *(Saisettia oleae)* Indian wax scale *(Ceroplastes sinensis)*	Observation of presence in leaves and twigs. **Threshold:** The first generation will be treated up to L3 when 3 individuals / outbreak are exceeded and in the 2nd generation, when 100% of eggs are reached.	
Aphids Cotton aphid *(Aphis gossypii)*	Chromotropic traps Observation of the percentage of damaged outbreaks. **Threshold:** Treatments are recommended when 25% of outbreaks are attacked. They will be	**Maintenance and improvement of vegetal covers and other habitats for wildlife reserves.** The covers of gramineas can significantly improve the effectiveness of the

Spirea aphid *(Aphis spiraecola)*	considered attacked shoots, shoots with the presence of aphids.	biological control that *Scymnus* and cecidomids mainly exert. **An efficient natural control can be observed by predatory entomophageal fauna such as:** **-** Coccinellids highlighting those of the genus *Scymnus*. - *Chrysoperla carnea* chrysopid neuropteran. - Dipteran like cecidomids *Aphidoletes aphidimyza*. **Use of authorized organic products, biocides or natural preparations:** **-** Potassium soap (1-3%) - Paraffinic and mineral oil (1-2%) - Rock dust mineral - Azadirachtin - Pyrethrin
Mediterranean fruit fly (*Ceratitis capitata*)	There are two types of sampling: a) Determine the population level through the use of food and sexual traps b) Determine the presence of the first chopped fruits; For this, 8 fruits of definitive size per tree will be observed. **Threshold:** a) In food traps: catches of 0.5 flies /flycatcher/day, before the envero, indicate the need for some treatment. b) Likewise, the presence of chopped fruit is significant. Especially, extra-early clementine varieties should be monitored, at the end of summer, and late orange varieties, at the end of the campaign (end of spring).	**Maintenance and improvement of vegetal covers and other habitats for wildlife reserves.** **Currently, the action of natural enemies is not enough to completely control the damage caused by *C. capitata.*** **Parasitoids** Three species of Braconidae have been imported since 2002: ***Diachasmimorpha tryoni*** (Cameron) and ***D. longicaudata*** (Ashmead) (larval parasitoids) and ***Fopius arisanus*** (Sonan) (egg parasitoid). So far, the two species of the genus ***Diachasmimorpha*** have been released in the field, without their acclimatization to our area being confirmed for now. Among the native parasitoids the most abundant are the pteromalidae: ***Pachycrepoideus vindemmiae*** (Rondani) y ***Spalangia cameroni*** Perkins. Both parasitizing pupae. **Use of authorized organic products, biocides or natural preparations:** Mass capture with flycatchers and traps with food attractants: -Mix: 2% ammonium acetate (or phosphate / bisphosphate) + a standard solution of hydrolyzed protein * (0.3%) + 1 tablespoon of borax.

		-Mix beer or 25% vinegar + biamonic phosphate + a standard solution of hydrolyzed protein * 0.3%. + 1 spoon of borax. -Mix beer or 25% vinegar + biamonic phosphate + a standard solution of hydrolyzed protein * 0.3%. -Commercial fishing boats with sexual attractants and putrescine (attractant that simulates synthetic food bait). Patching with Spinosad (0.2%) together with food attractant (hydrolyzed protein) and azadirectin, rotenone or pyrethrin can be added.
Woolly whitefly (*Aleurothrixus floccosus* Mask) Paraleyrodes (*Paraleyrodes minei*)	Sample in the summer (July) and fall (September-October) sprouting. Observe outbreaks its population and level of parasitism. Cottony whitefly has a preference for young shoots, so it is recommended to observe the presence of fly individuals (adults, eggs and nymphs) in these outbreaks. Thresholds: It is advisable to exercise some type of action against the pest if the level of infestation of the pest exceeds 20% of attacked outbreaks and the parasitism rate is less than 60%.	**Maintenance and improvement of vegetal covers and other habitats for wildlife reserves.** **Various species of predators can exert a population reduction of the pest: Coccinellids: *Clistotethus arcuatus and Cryptolaemus montrouzieri*, Neuropteran: *Chrysoperla carnea* and *Conwentzia psociformis.*** The parasitoid ***Cales noacki,*** that exerts total control of the pest since its introduction in our citrus fruits. **Use of authorized organic products, biocides or natural preparations:** **-** Potassium soap or neem oil
Leafminer (*Phyllocnistis citrella* Stainton)	In seedlings and grafts, the observation of attacked receptive shoots (100 shoots in 50 trees, 2 shoots / tree) in summer and autumn shoots is recommended. **Threshold:** In seedlings and grafts, treat when the presence of the leafminer is observed from the 2nd sprouting. It is important not to perform chemical applications on trees in full production.	**Maintenance and improvement of vegetal covers and other habitats for wildlife reserves.** **Predadors:** Many species of arthropods**:** ***Thrips* sp.** (Thysanoptera: Thripidae), ***Chrysoperla carnea*** (Neuroptera: Chrysopidae), ***Orius* sp.** (Hemiptera: Anthocoridae) and spiders (Araneae). Generally prey leafminer in the absence of the species that they usually consume. **Use of authorized organic products, biocides or natural preparations:** -Azadirachtin, neem, paraffinic oil, garlic extract, *B. thuringiensis* -
Citrus flower moth (*Prays citri*)	Flight monitoring with sex pheromones; observation of the phenology and degree of attack of flowers and fruits. **Thresholds:** It is advisable to intervene when there is more than 50% open flower and 5% of	No specific natural enemies are known. There is spontaneous predation by *Chrysopas* and other generalists. **Use of pheromone traps**

	damaged flowers and fruits are exceeded, or more than 10% of flower buds and buds are observed. Subsequent treatments if more than 5% of floral elements with live larvae are observed.	**Use of authorized organic products, biocides or natural preparations:** - Use of *Bacillus thuringiensis* to control the larvae of this lepidopteran.
Citrus Red Mite *Panonychus citri* (Mc Gregor)	Sampling every two weeks. Two fully formed leaves of the last sprout per tree should be sampled, determine number of leaves occupied by red mite. In addition, sample a mature leaf from the inside and determine the number of leaves occupied by phytoseids. **Threshold:** Treat when nº leaves with phytoseids < 30% and nº leaves with red mite>20% between august and october, >80% the rest of the year.	**Maintenance and improvement of vegetal covers and other habitats for wildlife reserves.** Especially grasses (Poaceae or Gramineae) **Natural biological control by predatory phytoseid mites:** *Euseius stipulatus, Neoseiulus californicus, Phytoseiulus persimilis and Typhlodromus* sp. **In the case of *Tetranichus urticae*, larvae and adults of the *Stethorus punctillum* coccinellid have been observed near their colonies.** **Use of authorized organic products, biocides or natural preparations:** -Use of paraffinic oil (1-1.5%) together with neem and aziradactin indica. -Use of garlic extract (repellent) - Use of wettable sulfur.
Red spider mite or two-spotted spider mite *(Tetranychus urticae* Koch)	Sampling with weekly or biweekly frequencies. Sampling is done by depositing two 56 cm Ø rings on the tree canopy and counting the number of "occupied rings", those that contain two or more symptomatic leaves (yellow spots). Four symptomatic leaves are also sampled and the number of leaves occupied by red spider is determined. **Threshold:** 10% of the leaves with presence of mobile forms or 2% of fruits with mobile forms in envero.	
Citrus bud mite (*Aceria sheldoni*)	For sampling, make two annual observations, in autumn and spring, before the new shoots reach 5 cm. Observe in four trees four complete branches (or 30 cm from the end) per tree. The branches will be of the last budding fully developed. In each branch observe: 1) visually in the field, presence of deformations 2) in the laboratory, at the binocular, presence of mites under the bracts of the buds. The intervention time will be when 25% of the branches show deformations or when the presence of mites is observed in 50% of the buds. If you decide to intervene, perform the application when most of the tree buds are between 4 and 6 cm.	
Mites *Eutetranychus* (*E. orientalis y E. banksi*)	To detect its presence in the field, it should be taken into account that, at the beginning of the colonization, the population is more abundant in the South-Southeast orientation of the tree and in the outer and upper part of the tree, it also has a preference for the central nerve of the beam of the leaves.	
Smaller green leafhopper (Empoasca)	Yellow chromotropic traps **Threshold:** Presence in traps	**Use of authorized organic products, biocides or natural preparations:**

		-Use of plant insecticide (pyrethrin, rotenone). -Use of hardeners: rock dust, horsetail, soda silicate. -Use of repellents: garlic extract, neem oil, nettle slurry extract.

*** Consult information about this in table 1 of section 1.3. Alternative host plants**

II. Management of pathogenic microorganisms

The approach we can have in organic citrus in the case of microorganisms is the same as in arthropods, but with more reason: to prevent rather than cure. The pathogenic microorganisms that cause disease are always difficult to manage once they have infested the tree; therefore, a preventive tactic is better. However, prevention must be based on the principles explained above, that is, on all types of cultural practices and diversity management before reaching products, which in these cases tend to be rather small in ecological agriculture (Porcuna Coto et al., 2010).

Among the nematodes, the only one that poses problems for citrus is *Tylenchulus semipenetrans*, a specific ectoparasite that grows in tired soils, with citrus repeats over citrus. *Poncirus trifoliata* and its hybrids (Citranges) create resistance to hypersensitivity, with the formation of a tissue under the bark that prevents larval penetration. **The most recommended actions are to establish rotations, resting the ground with fruit or vegetables. Of course, adding any type of organic matter (green manure, unfinished manure, etc.) that causes digestion in the soil before planting, can favor an increase in microorganisms, which would increase competition and would decrease the attack of pathogens.**

The brown rot of citrus fruit (Gomosis) is a disease caused by fungi of the genus *Phytophthora (Ph. Citrophtora, Ph. Parasitica*). These saprophytic fungi are found in any type of soil. Its damage occurs, especially in long periods of drought followed by waterlogging, causing anoxia (lack of oxygen) in the areas where it acts (around the neck of the tree). The symptoms go through a slight generalized yellowing, with the subsequent loss of leaves, deficiencies and dry of generalized branches, indicating that the damage has been installed in the neck and roots. **We can act using resistant varieties, such as bitter orange, citrange, Poncirus. With good drainage the chances of damage are reduced. It is advisable to perform watering by buckets, grooves or located (without touching the neck or graft), as well as avoid waterlogging or accumulation of water (do not leave water at the root for more than 2 hours).** The change to drip irrigation, if carried out without exhaustive control of soil moisture, usually causes many problems of excess water and waterlogging, with the consequent risk of infection of the fungus.

The Armillaria Root Rot (*Armillaria mellea, Dematophora necatrix (= Rosellinia necatrix, Clitocybe tabescens)* can be important at the time of transplantation or in new plantations. They are also facultative saprophytic fungi, which develop on old soil remains (dead roots, branches, etc.) Attacks can occur to the root system, by colonizing the woody parts of the thick roots and the base of the trunk. *Armillaria* affects more vigorous rootstocks (Citrange), where defoliation can be total in warm times; in the less vigorous (mandarins) the defoliation is small; the bitter orange tolerates it.This fungus endures with low O_2 contents, so it can be found more than 1 m deep. It should be avoided leaving the stumps on the ground, after starting the tree. It is advisable to remove the remains of vegetables or decompose them naturally (with rotations of cereals, green manures or fresh manure) for a enough period.

The anthracnose or dry branches (Colletotrichum gloesporoides) affects trees weakened by natural or water imbalances. It hardly occurs in mandarins and clementines. There are always aerial spores of the fungus. It spreads with the first autumnal rains. In more humid areas, it increases, causing a characteristic drying of young branches. We must control the weakness, lack of soil, compaction, irrigation, nutrition, etc. Treatments with cupric products can be performed, but it is not advisable for the accumulation of this heavy metal in the soil, and the disorders that this can cause.

The moldiness and rot of fruits are caused by fungi of the genera *Penicillium* (*P. italicum* or blue mold; *P. digitatum* or green mold) and *Phytophtora* (*Ph. Citrophthora*, Ph. Sp., watery or brown rot). They occur in plantation or post-harvest. The low nitrogen and moisture content of the skin, together with natural waxes, decrease the percentage of depreciation (they are more resistant skin). The molds penetrate the epidermis through wounds (from insects, rubs, etc.). They are important in storage and transportation. It is recommended to take care not to make wounds, eliminate or not harvest those seen and monitor the nitrogen fertilizer. Cupric compounds, prepared from microorganisms or natural antiseptic substances (essential oils, propolis) would be possible treatments in the field or postharvest.

The only notable bacteriosis is *Pseudomonas syringae*. It occurs in more humid areas, where citrus fruits are more delicate to this disease. In the autumn rains it enters by wounds of branches. **Copper and its derivatives can be healing in wounds. We can also perform treatments with whey and propolis.**

Viruses occur especially in weak or sensitive trees, in agroecosystems that are degenerating. In our case, before the entry of sadness, these diseases were hardly important. However, with the entry of resistant material to this virus (tolerant patterns), we began to have serious problems with other types of virosis and mycoplasmas. Sadness, psoriasis, tater leaf, Vein Enation-Woody Gal, exocortis, xyloporosis or Sttuborn, all have similar treatments. **The most recommended measure is to use certified, virus-free plants. Other hygiene measures are being careful with pruning tools and the like, which can serve as inoculum, scratching** and painting with propolis, cupric compounds or whey, which can stop some viruses (such as psoriasis or similar), or organic subscribers that also improve their health status (Porcuna Coto et al., 2010).

The main pathogenic organisms and their management can be summarized according to the following table, described by Porcuna Coto et al. (2010) for ecological control in Valencian citrus fruits.

Table 3. Most important diseases of Valencian ecological citrus, along with its ecological management (Porcuna Coto et al., 2010)

Phytopathogenic organism	Ecological Management Proposal
Nematodes ***Tylenchulus* sp.**	-Rotation with vegetables and fruit trees (7-10 years). -Use of tolerant patterns, for example: *Poncirus and Citrange.* -Potentiation of mycorrhizae and saprophytic fungi and soil antagonists (with organic matter and green manures). -Association with nematicidal plants (*Brassica* sp., *Sinapis* sp.). -Use of fresh manure buried in surface.
The brown rot of citrus fruit (Gomosis) is a disease caused by fungi of the genus *Phytophthora* (*Ph. Citrophtora, Ph. Parasitica*) and another fungis of soil, Armillaria Root Rot (*Armillaria mellea, Dematophora necatrix*)	- Enhancement of mycorrhizae and saprophytic fungi and soil antagonists. -Avoid waterlogging and irrigation in the neck: cultivation on plateau or with aerated wheels, monitor localized irrigation. -Solarization and biofumigation. -Gomosis biological control: *Mirotezium* sp., *Penicillium* sp. -Cleaning and disinfection of wounds with propolis, lime, sodium silicate, cupric compounds, potassium permanganate. Take care of replanting (rot), removing traces of roots, old branches, stumps, etc. Recommended rotation.
The anthracnose or dry branches (*Colletotrichum gloesporoides, Phomopsis citri, Phoma tracheiphilia o Diplodia mutila*)	- Watch, try only in the presence. -Avoid water or nutritional stress. - Disinfection with propolis, sodium silicate, potassium permanganate or copper. Soap can be mixed.
The moldiness and rot of fruits (*Phytophtora* sp., *Penicillum* sp.)	-In the field: keep the vegetation cover in the rainy season. -Avoid excess nitrogen. -Treat with permanganate or copper in skirts. -In stock: avoid injuries or blows, make a good mess.
Citrus tristeza virus (CTV) (Closteroviridae: Closterovirus)	-Use tolerant and free varieties (from controlled nurseries). -Avoid contacts with virotic materials (grafts). -Clean the pruning instruments well after each tree. -Eliminate adventures on which it can be transmitted.

According to what we have described in this section, regarding the management of arthropods and pathogenic microorganisms in ecological citriculture, we can summarize as a table, the phytosanitary products of possible use (Table 4). This summary has been based on a *"Guide of Phytosanitary Products of Possible Use in Organic Agriculture"* recently published by the Cabildo de Tenerife (Agrocabildo, 2019), which was prepared by consulting the registration of phytosanitary products of the Ministry of Agriculture, Fisheries and Food (Ministerio de Agricultura, Pesca y Alimentación-MAPA) updated as of September 18, 2019, choosing those that are considered, that can comply with the provisions of Annex II of EC Regulation No. 889/2008.

Table 4. Summary of Phytosanitary Products registered in Spain for possible use in ecological citriculture.

Denomination	Active ingredient/ Chemical Group	Tradename(s)	Pest or pathogen that controls
Substances of plant or animal origin			
Azadirachtin extracted from Azadirachta indica (Neem Tree)	Azadiractin 1% (like azadiractin A) [EC]	Adina	Caterpillars, aphids, red spider, whitefly, phyllocnistis, thrips, orthoptera, aphids, leaf miners, noctuids, nematodes, mealybugs, cicadellids, ceratitis, moths, lepidopteran larvae, diptera.
	Azadiractin 2,6% (like azadiractin A) [EC]	Azatin, Align, Zenith, Azar, Zafiro, Azafit	Aphids, whitefly, orthoptera, cicadellids, mealybugs, thrips, leafminers, ceratitis, moths, diptera, noctuidos, caterpillars, mealybugs, leafminer, nematodes, lepidopteran larvae.
Hydrolyzed proteins except gelatin	Hydrolyzed proteins 30% [SL]	Proteínas Hidrolizadas Life, Biocebo Attrack	Ceratitis, Olive fruit fly
		Nutrel	Mediterranean fruit fly, Olive fruit fly
Vegetable oils	Orange oil 6%[ME]	Limocide	Oidio fungus, whitefly, beetle, thrips, mites, cicadellids, **psyllid**, rust, fungal diseases, mildiu fungus, bedbugs.
	Orange oil 6% [SL]	Prevam	Aleyrodidae whitefly, cigarette, Thrips, Oidio and Mildiu fungus.
Natural pyrethrins extracted from	Piretrinas 4,65% (como extracto de pelitre) [EC]	Cordial Extra	Drosophila, whitefly, Aphids (*Myzus Persicae)*, thrips, moths, Cicadellids.

Chrysanthemun cinerariaefolium			
Pyrethroids (Deltamethrin or lambdacihalotrin only)	Deltametrin 0,015% [RB]	Decis Trap Ceratipack	Ceratitis
	Deltametrin 0,03% [RB]	Magnet Med	Ceratitis
Microorganisms or substances produced by microorganisms			
Microorganisms	*Bacillus thuringiensis* Aizawai 50% [WG]	Turex 50 WG	Prays, cluster moth, noctuidae, moth, leafminer.
	Bacillus thuringiensis Kurstaki 16% (16 mill. de U.I./g) [WP]	Presa 16, Bioscrop BT 16, Lepiback	Plusia, heliothis, prays, caterpillars, winding caterpillars, deflector caterpillars, orugueta, cluster moth.
	Bacillus thuringiensis Kurstaki (ABTS-351 strain) (32 mill. de CLU/g) 54% (540 g/kg) [WG]	Dipel DF	Caterpillars, scrubbing caterpillars, lizard, carpocaspa, caterpillar, prays, cacoecia, cluster moth
	Beauveria bassiana (ATCC 74040 strain) 2.3% (2,3X10E7 viable spores /ml) [OD	Naturalis-L	Aphids, whitefly, thrips, red spider, wireworms, fruit fly, cherry fly, olive fly, **psyllid**, tetranychid mite
	Trichoderma asperellum (ICC012 strain) 2% + *Trichoderma gamsii* (ICC080 strain) 2% (3 x 10E7 cfu/g (sum of both microorganisms) [WP]	Blindar	Esclerotinia, *Phytophtora*, brown rot, *Armillaria, Verticilium*, fungal wood diseases
	Trichoderma harzianum Rifai (T-22 strain) 1% (1 X 10E9 CFU/G) [WG]	Trianum P	Pythium, fungus deseases
Other substances			
Aluminum Silicate (Kaolin)	Kaolin 95% [WP]	Surround WP Crop Protectant	Fruit fly, prays, fly, **psyllid** (Apply preventively before laying eggs on fruits)
Copper compounds in the form of: Copper hydroxide, Copper oxychloride, Copper oxide, Bordeaux broth	Cupric hydroxide 13,6% (Expr. en CU) + Copper oxychloride 13,6% (Expr. en CU) [SC]	Airone, Airone SC Blue, Grifon	Bacteriosis, Mildiu, Anthracnosis, screening, Monilia, Alternaria, *Phytophthora* sp., shomopsis, spotted, tuberculosis, Cercospora.
	Cupric hydroxide 25% (Expr. en CU) [WG]	Boxer	Mildiu, brown rot, Monilia, screening, bacteriosis, spotted, tuberculosis, Anthracnosis, royas.

and Copper tribasic sulfate (Up to 6 kg of copper per ha and year. Notwithstanding the provisions, in the case of perennial crops, Member States may provide that the limit of 6 kg of copper may be exceeded during a given year, provided that the average amount actually used during a period of 5 years that covers this year plus the previous four years does not exceed 6 kg).			
	Cupric hydroxide 50% (Expr. en Cu) [WP]	Funguran-OH 50 PM	The brown rot of citrus fruit (Gomosis), mildiu, repilo, *Phytophthora* sp.
	Cupric hydroxide 50% (Expr. en Cu) [WP]	Hidroxigreen 50	Bacteriosis, *Phytophthora* sp., Alternaria, Anthracnosis, Mildiu, tuberculosis, *Alternaria* sp., roya.
	Cupric hydroxide 14% (exp. en Cu)+ Hidroxido cúprico 14% (exp. en Cu) [WG]	Cuprantol Duo	Mildiu, Leaf spot, *Phytophora infestans*, Pseudomonas, Phytophora sp., spotted, Anthracnosis.
	Cupric hydroxide 35% (exp. en Cu) [WP]	Cuprozin 35 WP	*Phytophthora* sp., mildiu, spotted
	Cupric hydroxide 38% (Expr. en Cu) [SC]	Curenox 38 Flow Blue	Bacteriosis, screening, mildiu, monilia, Alternaria sp., Anthracnosis, tuberculosis, roya.
	Cupric hydroxide 50% (Expr. en Cu) [WP]	Curenox 50 Blue, IQV Oxicloruro 50 Azul	Bacteriosis, Alternaria sp., Anthracnosis, mildiu, *Phytophthora* sp., spotted, phomosis, tuberculosis, roya, monilial, endophytic fungi, chancre.
		Covicampo-50, Cuprotec	*Phytophthora* sp., Anthracnosis, monilia, spotted, *Alternaria* sp.
		Cuper 50	*Phytophthora* sp., monilia, spotted, *Alternaria* sp.
	Cupric hydroxide 70% [SC]	Covinex 700 Flow, Cuper 70 Flow-Trade	*Phytophthora* sp., monilia, spotted, *Alternaria* sp., Phomisis, Anthracnosis, endophytic fungi, mildiu, bacteriosis.
Ethylene	Ethylene 4% [GA]	Frutargas	Growth regulator
		Nitroetil	Growth regulator, Suppression of regrowths
Paraffin oil	Paraffin oil 65,4% [EW]	Volck Verano, Benoil Naranjos, Agroaceite Blanco, Citrol, Aceite Emulsionable Agrofit	Aphids, whitefly, mealybugs, mites.

	Paraffin oil 79% [EC]	Benoil AE, Fulmit, Ivenol Massó, Ivenol-G, Ovipron, Cekuoil-V83, Citrol-Ina	Aphids, mealybugs, mites.
		Afroil-N, Laincoil FI	Mealybugs, mites, Red spider mite
	Paraffin oil 83% [EC]	Volck Miscible, Agroaceite Mosca	Whitefly, mealybugs, Aphids, mites.
Sulfur	Micronized sulfur 60%+ 4% Copper oxychloride (Expr. En CU) [DP]	Procuprico 60-4	Oidio

3.2.3. Biorational's products to control of psyllids in citrus

Biorational insecticides are substances that are derived from microorganisms, plants or minerals. Also, they can be synthetic substances similar or identical to others found in nature. These insecticides usually have a very low toxicity for humans and other vertebrates and degradates within a few hours after being applied. For these reasons, they are considered environmentally friendly. Its effect on wildlife and the environment is less harmful than that of conventional insecticides.

Among these biorational insecticides are: oils of vegetable or mineral origin (petroleum derived oils), botanicals that are extracted from plants (azadirachtin, essential oils, pyrethrin or pyrethrum, etc.), soaps (natural and synthetic), minerals (sulfur, kaolin or diatomaceous earth), growth regulators and pheromones.

Reduced-risk insecticides with novel chemistries and modes of action fulfill this requirement. Furthermore, application of reduced-risk insecticides in rotation with broad-spectrum insecticides may reduce the selection pressure for resistant alleles in target pest populations that is imposed by the latter compounds.

The insect growth regulators (IGRs) are known to be highly effective in killing immatures, especially nymphs, of several sucking insect pests, including ACP (Boina & Bloomquist, 2015)**.** Under field conditions, the protection offered by IGRs, (diflubenzuron, flufenoxuron, lufenuron, novaluron and pyriproxyfen) ranged from 3 days (diflubenzuron) to 4–6 weeks (lufenuron and flufenoxuron). **Given the potential of IGRs to reduce adult fecundity and control immatures, IGRs are an important and promising rotational tool in insecticide resistance management (IRM) programs for ACP** (Boina & Bloomquist, 2015).

Table 5. Potential biorational products to control of psyllids in citrus

3.2.4. Biological control of psyllids

Biorational product (recommended/used Doses)	Active ingredient/ Chemical Group	Psyllid(s) that controls and Biological(s) stage(s)	Results/Finding	Application	Reference(s)
Lovis petroleum spray oil (500 ml/100 l of water)	Mineral oil derived from petroleum	Effect of oil on ***Diaphorina citri:*** eggs and nymphs	When the oil concentration increased from 0.25% to 1.0% there was a linear decrease in the number of psylla of each stage present on foliar shoots after 8 days. 1st± 2nd instars were the most susceptible and eggs being the most tolerant to oil. It was clearly demonstrated that oil also reduced the resultant psylla populations when psylla free trees were sprayed prior to exposure to adult psylla (presumably the result of oviposition deterrence).	Authorized application in China, Asia, Australia, Florida and California	Rae, et al. (1997)
D-C-Tron NR petroleum spray (500 ml/100 l of water)	Mineral oil derived from petroleum	Effect of oil on *D. citri:* eggs and nymphs			
Guangdong petroleum spray oil (500 ml/100 l of water)	Mineral oil derived from petroleum	Effect of oil on ***Diaphorina citri:*** eggs and nymphs			
Spintor (*2 SC- 6 oz) (**Spinosad**, naturally obtained insecticide)	Insecticide of microbial origin. It is a toxin produced by the bacteria *Saccharopo-lyspora spinosa.*	Efect of ***Diaphorina citri:*** nymphs	Spintor provided initial reduction of ACP through 4 DAT with sustained activity against nymphs 8 DAT.	Florida	Childers & Rogers (2005)
Azadiractin 3.2%	Oil that is extracted from the seeds of the neem tree, *Azadirachta indica.*	***Trioza erythreae***	Authorized in organic farming. It is recommended making the applications early in the morning or before dusk, in the presence of first stages of the pest. Repeat it if necessary at intervals of 7 days.	Spain	https://www.phytoma.com/sanidad-vegetal/avisos-de-plagas/psila-africana-de-los-citricos-trioza-erythreae
Azadiractin 4.5%	Oil that is extracted from the seeds of the neem tree,	***Diaphorina citri***	**Effect on Adults:** adult psyllids showed a slight but significant repellent behaviour to treated plants, but	United States	Weathersbee & McKenzie (2005)

	Azadirachta indica.		showed no oviposition preference to treated or untreated plants. **Effect on Psyllid nymphs:** they were susceptible to azadirachtin at very low concentrations as a result of developmental inhibition. **At a concentration of 22.5 ppm azadirachtin, ecdysis was not observed past 4 days after treatment and all nymphs died within 7 days.** The densities of psyllid nymphs were significantly reduced by concentrations as low as 10 ppm of azadirachtin. **The product caused no phytotoxicity** to tender foliage of either citrus or orange jasmine plants. All these determine suitability of neem-based biopesticides for inclusion in citrus integrated pest management programs.		
Program combinated : Kaolim – Azadiractin-cotton seed oil- alcohol ethoxylate	**Azadiractin:** Oil that is extracted from the seeds of the neem tree, *Azadirachta indica.* **Caolín (Kaolin)** or Chinese clay, fine white clay powder. Mineral Insecticide	***Diaphorina citri***	Program on sequential applications of: Kaolim-azadiractin (neem oil), Kaolim-cotton seed oil-alcohol ethoxylate and adjuvants and Kaolim-aceite (cotton seed oil) This biopesticides combination showed high efficacy in controlling ACP under	Texas, US	Raygoza, J. (2010)

	Cotton seed oil: vegetable oil (cotton)		differents developmental stages. No long term residual control was nevertheless observed.		
Program combinated : Neem oil-alcohol ethoxylate and vegetable oil		***Diaphorina citri***	Program based on sequential application of: Neem oil, alcohol ethoxylate and adjuvants and vegetable oil. This program also highly effective for control of ***D. citri*** eggs and nymphs.	Texas, US	Raygoza, J. (2010)
Program combinated the application of plants extracts: Capsicin, garlic oil and garlic juice	**Vegetable oils and juice**	***Diaphorina citri***	Program solely based on application of Capsicin, garlic oil and garlic juice. Effective for adult control but less effective for nymph control	Texas, US	Raygoza, J. (2010)
The insect growth regulators (IGRs)	Insect growth regulators The mode of action of these IGRs is chitin biosynthesis inhibition (benzoylphenyl ureas)	***Diaphorina citri***	The insect growth regulators (IGRs) are known to be highly effective against immatures, especially nymphs, of several sucking insect pests, including ACP. Under field conditions, the protection offered by IGRs, (diflubenzuron, flufenoxuron, lufenuron, novaluron and pyriproxyfen) ranged from 3 days (diflubenzuron) to 4–6 weeks (lufenuron and flufenoxuron).	USA, Pakistan, Irán, India	Boina & Bloomquist (2015)
	Diflubenzuron (Micromite 80W DG)			USA	
	Flufenoxuron (Cascade 5-10C)			Pakistan Irán	
	Lufenuron (Match 050 EC)			Pakistan	
	Novaluron (Rimon 10EC)			India	
	Pyriproxyfen (Knack 0.86 EC)			USA	
HMOs (nC24 HMOs (0.35% v/v)	HMO (horticultural mineral oil)	***Diaphorina citri***	HMOs, petroleum oils and other oils have been used successfully to provide protection from ACP in the field	Malaysia	Boina & Bloomquist (2015)

Petroleum oils (Petroleum spray oil)			that was comparable or even better than conventional insecticides.		
	HMO (horticultural mineral oil)	***Diaphorina citri***	Application of some insecticides with HMO improved their performance against ACP. Significant suppression of adults prolonged 7–14 days and improved 9–47% with the addition of an adjuvant to treatments of fenpropathrin (Danitol 2.4 EC), sulfoxaflor (Closer SC), spinetoram (Delegate WG) and diflubenzuron (Micromite 80 WGS). HMO by itself provided an average of 36% reduction in adults for 18 days	Florida	Qureshi, Kostyk & Stansly (2014)

3.2.4.1. Natural enemies (Biological Control) of vector *Trioza erytreae* (African citrus psyllid)

ACT populations are often suppressed by generalist predators (Aubert, 1987; EPPO, 2014). About 50 predator species on citrus and other Rutaceae in Republic of South Africa (RSA) have been documented (Van den Berg et al., 1987), though such information is generally lacking for East Africa and specifically for Kenya. **In Réunion, two parasitoids have been credited with up to 75% parasitism in ACT but in RSA, their efficacy is limited by hyperparasitoids** (McDaniel and Moran, 1972; Van der Merwe, 1923). **According to Catling (1970), predators such as lacewings, syrphids, coccinellids, and predatory mites can suppress ACT populations**.

***Trioza erytreae* is highly parasitized by several species of hymenopteran parasitoids.** Parasitism rates were high in all the sampled areas in spring and ranged between 0.40 and 0.70. These rates are similar to those reported by Van der Merwe (1923) and Catling (1969) in South Africa and Swaziland, respectively, and Tamesse et al. (2009) in Cameroon. Therefore, as demonstrated by Catling in the 1960s, parasitoids are important biotic regulators of *T. erytreae* in those areas where insecticides are not sprayed in South Africa, e.g. abandoned and experimental orchards and public gardens (Catling, 1969, 1970). This result reinforces the suggestion of introducing exotic parasitoids to those areas where *T. erytreae* has arrived and where effective native parasitoids are absent. This is the case of Maderia (Portugal), the Canary Islands (Spain) and, more recently, mainland Europe (Cocuzza et al., 2016). **Among the species of primary parasitoids of *T. erytreae*, *T. dryi* is the most effective and abundant, as has been previously demonstrated by other studies in South Africa and Swaziland**

(Catling, 1969; Mc Daniel & Moran, 1972). Its relative abundance was higher than 90% in two sites (Perez-Rodriguez, et al., 2019).

Table 6. Natural enemies of vector *Trioza erytreae* (African citrus psyllid) and other control alternatives for integrated management.

Natural Enemies	Type (Pred/Par/Entomo)*	Stage of Host	Results/Finding	CSA*	Source/ Reference and Location
Adalia bipunctata (Coleoptera, Coccinellidae)	**Predator**	**Eggs**	It has been identified in citrus orchards in the Canary Islands associated to *T. erytreae* (Cocuzza et al., 2016). As observed by promptly predating eggs of *T. erytreae*, without observing more predation when different stages of *T. erytreae* were offered in a controlled environment for 48 hours (Hristova, 2014). Estevez et al. (2018) reported the predation of eggs by this coccinellid, in a timely manner, during their study to determine the population dynamics of *T. erytreae* and identify the natural enemies of this vector in the Canary Islands.	**CA**	**Hristova Gueorguieva (2014)** **Cocuzza et al. (2016)** **Estévez, et al. (2018)** Canary Island
Brumus quadripustulatus (Coccinellidae)	**Predator**	**Nymphs**	It has been identified in citrus orchards in the Canary Islands associated to *T. erytreae* (Cocuzza et al., 2016). Only ocassional predation on late nymphs stages was observed under controlled environmental conditions (Hristova, 2014)	**CA**	**Hristova Gueorguieva (2014)** **Cocuzza et al. (2016)** Canary Island
Allograpta pfeifferi (Diptera)	**Predator**	**Nymphs**	This predator consumed nymphs of *T. erytreae*, only when no other food resources available and after 1 - 2 days	**CA**	**Hristova Gueorguieva (2014)** Canary Island

Cheilomenes propinqua (Coleoptera)	**Predator**	**Nymphs Adults**	This predator showed the same preference for aphids as for nymphs of *T. erytreae*.	**CA**	**Hristova Gueorguieva (2014)** Canary Island
Chilocorus nigritus* (F.) *(Coleoptera, Coccinellidae)	**Predator**	**Nymphs**	It has been observed occasionaly feeding on psyllid's eggs and nymphs.	**CA**	**Cocuzza et al. (2016)** South Africa
***Chrysopa pudica* Nava (Neuroptera, Chrysopidae)**	**Predator**	**Nymphs**	Generalist predators of the African psyllid	**CA**	**Cocuzza et al. (2016)** South Africa
Chrysoperla carnea (Neuroptera, Chrysopidae)	**Predator**	**all immature stages and Nymphs**	*Chrysoperla carnea* has been observed depredating on all stages of *T. erytreae*, except adults because their mobility allows them to escape. It seems to present a preference for late nymphs, reaching consumption levels of 20 late nymphs (stages 4 and 5) in less than 48 hours (Hristova, 2014). It has been identified in citrus orchards in the Canary Islands associated to *T. erytreae* (Cocuzza et al., 2016) Estevez et al. (2018) reported this neuropter frequently predating all immature stages of the psila, during their study to determine the population dynamics of *T. erytreae* and their natural enemies in the Canary Islands	**CA**	**Hristova Gueorguieva (2014)** **Cocuzza et al. (2016)** **Estévez, et al. (2018)** Canary Island
Cryptolaemus montrouzieri (Coccinellidae)	**Predator**	**Eggs**	According Hristova (2014) timely predation of eggs of T. *erytreae* was observed. No predation was nevertheless registered on nymphal stages under a controlled environment for 48 hours (Larva of *C. montrouzieri* attacked by larva of *C. carnea* and by the mite *Leptus* sp).	**CA**	**Hristova Gueorguieva (2014)** **Cocuzza et al. (2016)** **Estévez et al. (2018)** Canary Island

			It has been identified in citrus orchards in the Canary Islands associated to *T. erytreae* (Cocuzza et al., 2016) Estevez et al. (2018) reported egg predation by this coccinellid in the Canary Islands.		
Exochomus quadripustulatus (L) (Coccinellidae)	**Predator**	**Nymphs**	In commercial crops of sweet orange trees from Tenerife and Gran Canaria, the predation of nymphs of *T. erytreae* by *Exochomus quadripustulatus* (L.) was observed in a timely manner.	**CA**	**Estévez et al. (2018)** Canary Island
Episyrphus balteatus (Diptera, Syrphidae)	**Predator**	**all immature stages**	The presence of this syrphid was observed and identified, although it was not confirmed preying on *T. erytreae* (Hristova, 2014). Estevez et al. (2018) reported this syrphid as a frequent predator of all immature stages of the psila in the Canary Islands	**CA**	**Hristova Gueorguieva (2014)** **Estévez et al. (2018)** Canary Island
Delphastus catalinae (Horn) ***(Coccinellidae)***	**Predator**	**Eggs**	Estevez et al. (2018) reported predation of eggs of psila by this coccinellid in the Canary Islands.	**CA**	**Estévez et al. (2018)** Canary Island
Harmonia axyridis (Coccinellidae)	**Predator**	**Eggs** **Nymphs**	According to Hristova (2014), ocassional predation of eggs and late nymphs of *T. erytreae* was registered, both during the observation of field samples and the laboratory experiments of predation. It differs from the rest of coccinellids in that it is more aggressive. It has been identified in citrus orchards in the Canary Islands associated	**CA**	**Hristova Gueorguieva (2014)** **Cocuzza et al. (2016)** **Estévez et al. (2018)** Canary Island

			to *T. erytreae* (Cocuzza et al., 2016) Estevez et al. (2018) reported that adults and larvae of this coccinellid were frequent predators of eggs of the psila in the Canary Islands.		
Iphiseius degenerans (Berlese) **(*Acari, Phytoseiid*)**	**Predator**	**Nymphs eggs**	Ocassional predator with feeding activity on psyllid eggs and nymphs. It has been observed attacking eggs and nymphs of *T. erytreae.*	**CA**	**Hristova Gueorguieva (2014)** **Cocuzza et al. (2016)** Canary Island South Africa
Leptus sp.* *(Acari, Erythraeidae)	**Parasite**	**Nymphs Adults**	According to Hristova (2014), larvae of *Leptus* sp. were observed parasitizing nymphs and adults of *T. erytreae* (having up to 10 larvae per nymph). All the collected individuals were in larval phase, not finding any adult. The larvae of the mite were "hooked" by the chelycer mainly to the abdomen of the psila, allegedly sucking the hemolymph.	**CA**	**Hristova Gueorguieva (2014)** **Cocuzza et al. (2016)** Canary Island
***Microtus sjoestedti* Wheel**	**Predator**	**Nymphs**	Generalist predators of the African psyllid	**CA**	**Cocuzza et al. (2016)** South Africa
Mites belonging to the genera ***Bochartia*** and ***Abrolophus***	**Predator**	**Eggs Nymphs**	Ocassional predator feeding activity on psyllid eggs and nymphs.	**CA**	**Cocuzza et al. (2016)** South Africa
Olla v-nigrum (Mulsant) *(Coccinellidae)*	**Predator**	**Nymphs**	Estevez et al. (2018) reported the predation of nymphs by this coccinellid in the Canary Islands.	**CA**	**Estévez, et al. (2018)** Canary Island
Orius laevigatus* (Fieber) *(Anthocoridae)	**Predator**	**Eggs Nymphs**	It was observed promptly by predating nymphs of *T. erytreae*. The larval phase of *O. laevigatus* has been observed making an attempt at predation, because it only stung nymphs without sucking (Hristova, 2014).	**CA**	**Hristova Gueorguieva (2014)** **Cocuzza et al. (2016)**

			It has been identified in citrus orchards in the Canary Islands associated to *T. erytreae* (Cocuzza et al., 2016) Estevez et al. (2018) reported the predation of eggs by this anthocorid in the Canary Islands.		**Estévez, et al. (2018)** Canary Island
Anthocoris* sp. *(Anthocoridae)	**Predator**	**Eggs**	Estevez et al. (2018) reported the predation of eggs by this anthocorid, in the Canary Islands.	**CA**	**Estévez, et al. (2018)** Canary Island
***Psyllaephagus pulvinatus* Waterston (Hymenoptera, Encyrtidae)**	**Parasite: Primary parasitoid. Solitary koinobiont endoparasitoid**	**Nymphs**	In Cameroon, as in South Africa, *P. pulvinatus* is found among the main primary parasitoids. It is a predominant specie in Cameroom (Tamesse, 2009). It has been reported parasitizing nymphs of *T. erytreae*. It nevertheless is frequently attacked by a complex of hyperparasitoids It was more efficient than *T. dryi* in Cameroon, suggesting that the efficacy of the parasitoid may vary depending on the environment.	**CA**	**Tamesse, J.L. (2009) Hristova Gueorguieva (2014)** **Cocuzza et al. (2016)** **Perez-Rodriguez, J., Kruger, K., et al. (2019)** South Africa, Swaziland and Cameroom
Syrphophagus cassatus (Hymenoptera)	**Secondary parasitoid**		In Cameroon, as in South Africa, S. *cassatus* is found among the main secondary parasitoids. It is a predominant specie in Cameroom (Tamesse, 2009).	**CA**	**Tamesse, J.L. (2009)** Cameroom
Tamarixia dryi* (=*Tetrastichus dryi)* (Waterston) *(Hymenoptera, Eulophidae)	**Parasite: Primary parasitoid. Solitary koinobiont ectoparasitoid**	**Nymphs**	It has been reported as the most common and effective parasitoid of *T. erytreae* (Specific endoparasite). *T. dry* is the organism that induces the highest mortality rates in the population of *T. erytreae,* although it is frequently	**CA BCD**	**Aubert et al. (1980) Tamesse, J.L. (2009) Hristova Gueorguieva (2014)** **Estévez, et al. (2018)**

			attacked by a complex of hyperparasitoids In experiences of biological control of *T. erytreae*, the best results have been obtained through classical biological control, with the introduction of this specific parasitoid. On the island of Reunion, *T. erytreae* is considered eradicated since 1980 due to the high percentage of parasitism obtained after the releases of *T. dryi* (Aubert et al., 1980). In Cameroon, as in South Africa, *T. dry* is found between the main primary parasitoids, is a predominant specie in Cameroom. Is a predominant specie in Cameroom (Tamesse, 2009). Currently, in the Canary Islands, an introduction program of the *Tamarixia dryi* parasitoid has been launched for mass rearing and subsequent release on the islands (Estévez et al., 2018). *Tamarixia dryi* was the most abundant primary parasitoid in Pretoria and Nelspruit (South Africa) (>95% of the emerged parasitoids), according Perez-Rodriguez et al. (2019)		**Urbaneja-Bernat, et al. (2019)** **Perez-Rodriguez, J., Kruger, K., et al. (2019)** Cameroom Canary Island, South Africa, Reunion Island, Mauritius, Swaziland
***Tamarixia* sp. (*Hymenoptera, Eulophidae*)**	**Parasite: Primary parasitoid.**	**Nymphs**	Globally, the best results in the control of HLB vectors have been through biological control through the introduction of specific parasitoids belonging to the *Eulophidae* family, genus Tamarixia.	**CA BCD**	**Tamesse, J.L. (2009)** **Perez-Rodriguez, J., Kruger, K., et al. (2019)** Cameroom South Africa

			Tamarixia sp. (65%) was one the most abundant species in Nelspruit and Tzaneen (South Africa) according Perez-Rodriguez et al. (2019)		

***Type of NE:** Predator (Pred), Parasite (Par), Entomophatogenic (Entomo)
***Current state of application of the natural enemy (CSA):** Research (Re), Cited application (CA), Biological control demonstrated with results in the field (BCD)

3.2.4.2. Natural enemies (Biological Control) of vector *Diaphorina citri* (African citrus psyllid)

Miranda et al. (2008) **described as natural enemies of *D. citri* the predators *Cycloneda sanguinea* (L.), *Chilocorus cacti* (L.), *Exochomus cubensis* Dimn, *Scymnus distinctus* Casey (Coleoptera: Coccinellidae), *Chrysopa* sp. (Neuroptera: Chrysopidae) and *Ocyptamus* sp. (Diptera: Syrphidae).** In addition, **among the most important species of the coccinelidae family that have been described as predators of *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), we find *Olla v-nigrum* (Mulsant), *Axion* sp., *Arawana* sp., *Azya orbigera* (Mulsant) and *Brachiacantha decora* (Casey)** (Michaud, 2001, 2002, 2004; González et al., 2010).

Generally, there are numerous predators attacking *D. citri* populations. Usually the most abundant are some species of lacewings of Chrysoperla and Ceraeochrysa gender, also coccinellid (Cortez-Mondaca et al., 2010; Lopez-Arroyo et al., 2010; Cortez-Mondaca et al., 2016). Recently, Khan et al. (2016) reported the ladybird *Adalia bipunctata* L. (Coleoptera: Coccinellidae) as a potential predator of *D. citri*.

Regarding parasitoids, the ectoparasitoid *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) and the endoparasitoid *Diaphorencyrtus aligarhensis* (Shafee, Alam and Argarwal) (Hymenoptera: Encyrtidae) are accepted as the only known primary parasitoids of *D. citri* (Grafton-Cardwell et al., 2013).

Tamarixia radiata is an idiobionte specific ectoparasitoid of nymphs of the Asian citrus psyllid, which was described from specimens that emerged from parasitized nymphs of *D. citri* on lemon leaves collected in Pakistan (Waterston, 1922). *T. radiata* has been successfully introduced to control *D. citri* in Reunion, Taiwan, Mauritius, the Philippines, Saudi Arabia, Indonesia (East Java), Guadeloupe and the United States of America (Florida) (Grafton-Cardwell et al., 2013).

From 2014–2015, experimental *D. citri* cohorts were monitored by Kistner et al. (2016) to determine survivorship, life table parameters, and marginal rates of mortality of immature *D. citri* at three sites in Riverside County, at South of California. The egg through adult emergence rates were used to assess the relative importance of biotic and abiotic factors on psyllid survivorship rates by life stage. **The predation of immature stages of *D. citri* by larvae of *Allograpta* sp. (Diptera: Syrphidae) and *Chrysoperla* sp. (Neuroptera: Chrysopidae) comprised 86% of all**

observed predation mortality. When protected from all other arthropods, parasitism by *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) comprised 21% of the total marginal rate of immature *D. citri* mortality. Overall, ***D. citri* net reproductive rates were reduced by 55–95% when exposed to natural enemies, indicating the importance of the classical biological control agent, *T. radiata*, and generalist predators in reducing *D. citri* densities in urban areas of southern California but also in commercial citrus groves of Florida** (Qureshi et al. 2009; Monzo et al. 2014; Kistner et al., 2016).

In some places like California, the top priority for citrus industry has been to diminish the rate of bacterium spread, by reducing Asian citrus psyllid populations in urban areas, where this pest primarily resides. In such as sense, according Milosavljević et al. (2017), an **alternative approach has been a classical biological control program using parasitoids, *Tamarixia radiata* and *Diaphorencyrtus aligarhensis*, which attack the psyllid nymphs**. *D. aligarhensis* is a host-specific solitary endoparasitoid of second through fourth instar ACP nymphs, Like *T. radiata*, this parasite kills its hosts through a combination of parasitism and host feeding, and a single *D. aligarhensis* female can kill up to 280 nymphs in her lifetime (Rohrig et al. 2011).

Moreover, the combination of *T. radiata* and increased attacks by native predators, such as syrphid fly larvae, which have started using ACP nymphs for food, are having a substantial impact, reducing urban ACP populations by more than 90% at some locations at certain times of year. In California, *T. radiata releases* are largely restricted to urban-grown citrus; releases are not made in commercial citrus production areas that are under area-wide management, where insecticide applications are coordinated over large areas and spray residues cause substantial mortality of *T. radiata* (Hall & Nguyen, 2010; Milosavljević et al., 2017). However, in Florida*, T. radiata* have established widely (Hoy & Nguyen, 2001), but their impact on ACP is not high, especially in comparison to coccinellid predator species, which appear to be significantly regulating ACP populations (Michaud, 2004). The reasons for the putative poor performance of *T. radiata* are varied, and may include the parasitoid's high sensitivity to pesticide residues in commercial citrus groves, low levels of genetic variation in released parasitoids that may have reduced their fitness and subsequent efficacy, and interference by ants tending honeydew-producing ACP nymphs (Navarrete et al., 2013; Milosavljević et al., 2017).

It is important to account the impacts of generalist natural enemies and invasive ants. Exchanges between natural enemies and ants that disrupt natural enemy activity are highly relevant interactions affecting biocontrol success. Ants are widely recognized as natural enemy antagonists because of the food-for-protection mutualisms they form with honeydew-producing hemipterans (HPHs), like ACP. Many species of HPH are invasive, economically damaging pests (e.g., aphids, mealybugs, scales, whiteflies and psyllids) (Helms, 2013). In addition to directly protecting HPHs from natural enemies, ants disperse tended pests to new foraging areas and provide sanitation services to HPHs, which reduce the incidence of disease and honeydew drowning. Furthermore, tended HPHs may respond to ant presence by increasing their rate of phloem ingestion, resulting in more rapid development and higher reproductive output (Yoo & Holway, 2011). Because ants may exacerbate existing pest problems, ant control is a critical component of integrated pest management (IPM) programs targeting HPH pests, particularly programs that rely on biological control agents for population suppression. Studies from Florida found that ACP parasitism by *T. radiata* was substantially higher in groves where ACP-tending ant species were controlled (Navarrete et al. 2013; Milosavljević et al., 2017).

Table 7. Natural enemies (Biological Control) of vector *Diaphorina citri* (Asian citrus psyllid) and other control alternatives for integrated management.

Natural Enemies	Type (Pred/Par/Entomo)*	Stage of Host	Results/Finding	CSA*	Source/ Reference and Location
***Allograpta* sp. (Diptera: Syrphidae)**	**Predator**	**Inmmature stages**	The predation of immature stages of *D. citri* by larvae of *Allograpta* sp. (Diptera: Syrphidae) comprised 86% of all observed predation mortality.	**CA**	**Kistner et al. (2016)** California
***Anystis baccarum* (L.) (Acari) Whirligig mite**	**Occasional predator**	-	-	**CA**	**(Yang et al., 2006)** cited in **Hall (2008)** China
***Harmonia axyridis Pallas* (Coleoptera: Coccinellidae)**	**Predator**	**Nymph**	-	**CA**	**Michaud and Olsen (2004)** cited in **Michaud (2004)**
***Azya Orbigera* (Coleoptera: Coccinellidae)**	**Predator**	-	-	**CA**	**Preza Durán (2011)** Mexico (Veracruz)
***Brachiantha dentipes* (Coleoptera: Coccinellidae)**	**Predator**	**Adults**	-	**CA**	**Michaud (2004)** Florida
***Brachiantha decora* (Coleoptera: Coccinellidae)**	**Occasional predator**	-	-	**CA**	**Preza Durán (2011)** Mexico (Veracruz)
***Brachiacantha testudo* (Coleoptera: Coccinellidae)**	**Occasional predator**	-	-	**CA**	**Murillo-Cuevas et al. (2010)** Mexico
***Brumus suturalis* (Fab.) (Coleoptera: Coccinellidae)**	**Generalist predators**	-	-	**CA**	**Hall (2008)** India

***Cheilomenes sexmaculata* Fab. (Coleoptera: Coccinellidae)**	**Generalist predators**	**-**	**-**	**CA**	**Hall (2008)** India
***Chilocorus nigrita* (Fab.) (Coleoptera: Coccinellidae)**	**Predator**	**-**	Occasional predator of *D. citri*	**CA**	**Hall (2008)** **(Samways 1984)** cited in **Cocuzza at al. (2016)** India Florida
***Chilocorus cacti (L.)* (Coleoptera: Coccinellidae)**	**Generalist Predator**	**Egg**	González et al. (2003) reported percentages of predation of these insects in eggs of *D. citri*, but the presence of these natural enemies is sporadic, depending on high levels of population of *D. citri*	**CA**	**González et al. (2000; 2003)** Cuba **Miranda et al. (2008)** **Murillo-Cuevas et al. (2010)** MExico Florida
***Chrysopa boninensis* Okamoto (Neuroptera: Chrysopidae)**	**Predator**	**Nymph**	**-**	**CA**	**(Yang et al. 2006)** cited in **Hall (2008)** China Florida **Alemán et al. (2007)** Taiwan
Chrysopids *(Neuroptera: Chrysopidae)* -*Chrysoperla comanche* (Banks), -*Chrysoperla rufilabris* (Burmeister) -*Chrysoperla carnea* s. lat.	**Predator**	**Egg Nymph all immature stages**	According Kistner et al. (2016), The predation of immature stages of *D. citri* by *Chrysoperla* sp. comprised 86% of all observed predation mortality. According Cortez-Mondaca et al. (2019): ***Ch. comanche* and *Ch. rufilabris*** showed greater capacity for predation immature eggs, nymphs girls (nymph 1 and nymph 2) and large nymphs (nymph 4 and nymph 5) to ***D. citri*** during the first six hours of exposure prey to predator. In the last reading of predation, at 24 h, all species of lacewing consumption	**CA**	**(Aubert 1987)** cited in **Hall (2008)** Réunion and Nepal Florida **Murillo-Cuevas et al. (2010)** **Preza Durán (2011)** Mexico (Veracruz) **Cortez-Mondaca, et al. (2016)** Mexico **Kistner et al. (2016)** California

			had almost similar (p> 0.05), about 100 specimens per predator. Egg consumption and nymph girl was greater than that of large nymphs. These results support the use of ***Ch. comanche*** for biological control of ***D. citri.***		
Cycloneda sanguinea (L.) **(Coleoptera: Coccinellidae)**	**Generalist Predator**	**Nymph Egg**	Larvae and adult feed on the nymphs of *D. Citri,* is a very voracious predator. González et al. (2003) reported percentages of predation of these insects in eggs of *D. citri* between 33.3% and 41.4%, but the presence of these natural enemies is sporadic, with dependence on high levels of *D. citri* population.	**CA**	**González et al. (2003) (Michaud, 1999, 2002a)** cited in **Michaud & Olsen (2004) Hall (2008) Miranda et al. (2008)** Florida Cuba **Preza Durán (2011)** Florida Mexico (Veracruz)
Coccinella **rependa Thunberg (Coleoptera: Coccinellidae)**	**Generalist predators**	**-**	-	**CA**	**Hall (2008)** India
Coccinella septempunctata **L (Coleoptera: Coccinellidae)**	**Generalist predators**	**-**	-	**CA**	**Hall (2008)** India China
Coelophora inaequalis **(F.) (Coleoptera: Coccinellidae)** Ladybird	**Predator**	**Young nymph Adults**	Feed on the young *D. citri* larvae	**CA**	**Étienne, et al. (2001).** Guadeloupe islands **Michaud (2004)** Florida
Curinus coeruleus **Mulsant**	**Predator**	**Nymph**	Larvae and adult females feed on the nymphs of *D. citri*	**CA**	**Michaud & Olsen (2004) Hall (2008)** Florida
Diaphorencyrtus aligarhensis **(Hymenoptera: Encyrtidae)**	**Parasite: Endoparasitoid** (well-known parasitoid)	**Nymph**	*C. aligarhensis* is a host-specific solitary endoparasitoid of second through fourth instar ACP nymphs. In addition to acting as a parasite, feeds on young nymphs, showing death rates of	**CA**	**Étienne, et al. (2001) Skelly and Hoy (2004)** **Hall, E.G. (2008)** India, Philipine, Viet-nam, China,

			66% (Skelly and Hoy, 2004). Like *T. radiata*, this parasite kills its hosts through a combination of parasitism and host feeding, and a single *D. aligarhensis* female can kill up to 280 nymphs in her lifetime (Rohrig et al., 2011)		Guadeloupe islands, Réunion Island, Taiwan, Florida **(Rohrig et al., 2011) Milosavljević et al. (2017)** California
***Exochomus childreni* Mulsan (Coleoptera: Coccinellidae)**	**Predator**	**Nymph**	Larvae feed on the nymphs of *D. Citri,* is a very voracious predator.	**CA**	**Michaud & Olsen (2004) Hall (2008)** Florida
***Exochomus marginipennis* (Leconte) (Coleoptera: Coccinellidae)**	**Predator**	**Egg Nymph**	Under controlled conditions, the larva IV (98.8 ± 2.61) followed by the larva III (97.60 ± 3.43) and the adult (96 ± 2.60) exercised greater predation. The nymph V of D. citri is the stage consumed in less quantity by the adult and the four larval stages	**Re**	**Palomares-Pérez, et al. (2016)** Mexico
***Harmonia axyridis* Pallas**	**Predator**	**Nymph**	Larvae and adults feed on the nymphs of *D. citri.* Michaud and Olsen, (2004), determined that *H. axyridis* was the most voracious predator of the Asian psyllid.	**CA**	**Michaud & Olsen (2004) Halbert & Manjunath (2004) Hall (2008)** Florida
***Hibana velox* (Becker)** (spider)	**Predator**	**Nymph**	According Michaud (2004) was a notable presence as predator in Florida	**CA**	**(Michaud, 2004)** cited in **Hall (2008)** Florida
Olla abdominales	**Predator**	**-**	-	**CA**	**Reyes (2006)** Argentina
***Olla v-nigrum* Mulsant**	**Predator**	**Nymph**	Larvae and adult feed on the nymphs of *D. Citri,* is a very voracious predator.	**CA**	**Michaud (2001) Michaud, & Olsen (2004). Hall (2008) Preza Durán (2011)** Florida Mexico (Veracruz)
***Psyllaephagus diaphorinae* Lin & Tao**	**Primary parasite**	**Nymph**	**-**	**CA**	**(Viraktamath & Bhumannavar,**

					2002) cited in **Halbert & Manjunath (2004)** Philippines
Saprinus chalcites Illiger	**Predator**	**-**	**-**	**CA**	**(Al-Ghamdi 2000)** cited in **Halbert & Manjunath (2004)** Saudi Arabia
Species of *Scymnus (Coccinellidae)*	**Predator**	**-**	**-**	**CA**	**(Gravena et al. 1996)** cited in **Halbert & Manjunath (2004)** Brazil
Syrphid flies in the *genus Allograpta (Ex. Allograpta obliqua* Say*)*	**Predator**	**Nymph Adults**	Observed attacking *D. citri* and capable of completing development on a diet of *D. citri* nymphs.	**CA**	**(Aubert, 1987; Michaud 2002)** cited in **Hall (2008) Michaud (2004)** Réunion, Nepal, Florida
***Syrphophagus taiwanus* Hayat & Lin**	**Hyperparasite**	**Nymph**	**-**	**CA**	**(Viraktamath & Bhumannavar, 2002)** cited in **Halbert & Manjunath (2004)** Taiwan
***Tamarixia radiata* (Hymenoptera: Eulophidae)**	**Parasite:** develops as an **idiobiont ectoparasitoid** on ***D. citri*** larvae. (well-known parasitoid)	**Nymph**	***T. radiata*** attack the psyllid nymphs, and has established widely and, in combination with generalist predators. In Reunion Island, *T. radiata*, parasitized between 60% to 70% of the nymphs of *D. citri* (Aubert, 1987). According González et al. (2001) (in Cuba) parasitized the second, third and fourth nymphal stage of the Asian psyllid and concluded after three years of evaluation, they observed a control effectiveness between 30 and 97.3%, with the lowest	**BCD**	**Aubert (1987)** cited by **McFarland & Hoy (2001) Étienne, et al. (2001) González et al. (2002) Skelly & Hoy (2004) Pluke et al. (2005) Hall (2008) Postali et al. (2010) Preza Durán (2011) Milosavljević et al. (2017)** India, Guadeloupe Islands Mauritius, Réunion, Island, The United States (Florida, California and

			parasitism percentages in the month of June and the highest between November and December. *T. radiata*, is considered a solitary parasitoid, which in addition to acting as a parasite, feeds on young nymphs, showing death rates of 57% (Skelly and Hoy, 2004). In Puerto Rico, Pluke et al., (2005), determined that the parasitism of *T. radiata* was 70% in the last nymphal stages of the psyllid. This parasitoid was also found in *Murraya paniculata*, parasitizing nymphs of D. citri between 48% and 77% regulating the populations of the psyllid. According Milosavljević et al. (2017) the combination of *T. radiata* and increased attacks by native predators, such as syrphid fly larvae, which have started using ACP nymphs for food, are having a substantial impact, reducing urban ACP populations by more than 90% at some locations at certain times of year.		Texas), Cuba, Puerto Rico Taiwan, Brazil, Viet-nam, Puerto rico, Arabian Peninsula, Mexico (Veracruz).
***Ocyptamus sp.* (Diptera: Syrphidae)**	**Generalist Predator**	**Nymph Egg**	González et al. (2003) reported percentages of predation of these insects in eggs of *D. citri*, but the presence of these natural enemies is sporadic, depending on high levels of population of *D. citri*	**CA**	**Miranda et al. (2008)** **González et al. (2003)** Cuba

***Scymnus distinctus* Casey (Coleoptera: Coccinellidae)**	**Generalist Predator**	**Egg**	González et al. (2003) reported percentages of predation of these insects in eggs of *D. citri*, but the presence of these natural enemies is sporadic, depending on high levels of population of *D. citri*	**CA**	**González et al. (2003)** Cuba
***Zelus longipes* L.** (The milkweed assassin bug)	**Occasional predator**	**-**	**-**	**CA**	**(Hall et al. 2008)** cited in **Hall (2008)** Florida

***Type of NE:** Predator (Pred), Parasite (Par), Entomophatogenic (Entomo)
***Current state of application of the natural enemy (CSA):** Research (Re), Cited application (CA), Biological control demonstrated with results in the field (BCD)

3.2.5. Entomophatogenic fungi to control of psyllids

3.2.5.1. *Trioza erytreae*

In South Africa, the ***Cladosporium oxysporum* fungus**, which was multiplied and tested in the laboratory against *T. erytreae* with good results (Samways & Grech, 1986), was isolated from the pseudococcal *Planoccocus citri*. Likewise, it has been observed that ***Capnodium citri*,** which induces a blackish mold, commonly known as fumagina or bold, is capable of causing a fungal epizootic in *T. erytreae* in artificial breeding. However, the effects of the two fungi are dependent on density, which is not compatible with the control of low levels of plague, being frequent on natural conditions (Aubert, 1987).

Globally, several species of entomopathogens have been reported to attack ACT, with some of them used as biopesticides. Psyllids are known to be highly susceptible to a number of entomopathogenic fungi (Avery et al. 2009; Meyer et al. 2007;2008), **and among the species known to attack ACT are *Isaria fumosorosea* Wize, *Isaria javanica* Friedrichs & Bally, and *Hirsutella citriformis* Pat.** (Avery et al. 2009; Étienne et al. 2001) (Fordjour Aidoo, 2019).

Tabla 8. Entomopathogenic fungi for control ***Trioza erytreae* (African citrus psyllid)**

Type (Pred/Par/Entomo)*	Stage of Host	Results/Finding	CSA*	Source/ Reference and Location
***Cladosporium oxysporum* (Berk. & Curt.) Entomophatogenic fungus**	**Nymphs**	This entomopathogenic fungus has been multiplied and tested in laboratory against *T. erytreae* with good results.	**CA**	Grech and Samways 1985 cited in **Cocuzza et al. (2016)** South Africa

***Type of NE:** Predator (Pred), Parasite (Par), Entomophatogenic (Entomo)
***Current state of application of the natural enemy (CSA):** Research (Re), Cited application (CA), Biological control demonstrated with results in the field (BCD)

3.2.5.2. *Diaphorina citri*

Regarding entomopathogenic fungi, in 1987, Aubert cited by Halbert and Manjunath (2004), indicated that *Cladosporium* sp. and *Capnodium citri* Mont., could be a good alternative for the control of *D. citri*, when determining in the Reunion Island a mortality of nymphs between 60% and 70%, when there was a minimum diurnal relative humidity higher than 87.9%. Etienne et al., (2001), cited by Halbert and Manjunath (2004), noted that **it is common to observe the fungus *Hirsutella citriformis* Speare, controlling psyllids, when the relative humidity was greater than 80%** (Preza Durán, 2011).

Several species of entomopathogenic fungi that infect *D. citri* and that may be useful as biopesticides have been reported in the literature (Mellín-Rosas et al., 2016). **According to Kondo et al. (2015, 2017), among the most cited species can be included: *Isaria fumosorosea* Wize (=*Paecilomyces fumosoroseus*), *Hirsutella citriformis* Speare, *Lecanicillium* (= *Verticillium*) *lecanii* Zimm., *Beauveria bassiana* (Bals.) Vuill., *Cladosporium* sp. nr. oxysporum Berk. & MA Curtis, *Acrostalagmus aphidum* Oudem, *Paecilomyces javanicus* (Friederichs & Bally) AHS Brown & G Smith, and *Capnodium citri* Berk. & Desm.** (Hall et al. 2012; Mellín-Rosas et al. 2016).

In a research project carried out in Sinaloa, Mexico in 2009 and 2010, the entomopathogen mixture (***Metarrizium anisopliae, Beauveria bassiana and Paecilomyces fumosoroseus***) was sprayed at 10 mL / L-1 of water, **obtaining 73% and 87% of control of nymphs in 2009 and 2010, respectively** (Sandoval et al., 2010). Preza Durán (2011) was able to identify the fungus ***Hirsutella citriformis* with a high number of mycosed psilids in Persian lemon, with greater affectations in October and December, when the average temperature was 20 to 25 °C and the relative humidity remained at 80 %.** In this way, it coincides with that cited by Halbert and Manjunath (2004) **demonstrating that this entomopathogenic fungus is capable of regulating *D. citri* populations naturally and that its occurrence is common during periods where the relative humidity is greater than 80%** , which is decisive for its growth and sporulation.

Zhang et al. (2015) realized one study tests the novel use of predatory mites for dissemination of a fungal pathogen for insect biocontrol in the laboratory. They first **evaluated the pathogenicity of *Beauveria bassiana* at several spore suspension concentrations against the nymphs of both the Asian citrus psyllid (*Diaphorina citri*) and two predatory mite species (*Amblyseius swirskii* and *Neoseiulus cucumeris*).** The *B. bassiana* spores at suspension concentrations greater than 104 spores x ml^{-1} were highly effective against *D. citri* nymphs, resulting in a mortality approaching 100% after 7 days, but caused only low mortality rates of *A. swirskii* and *N. cucumeris* nymphs (15 and 10%, respectively) after 7 days. **They then observed whether these two predatory mites, when dusted with *B. bassiana* spores, could disseminate the pathogen to *D. citri* residing on small twigs of potted *Murraya paniculata* plants under high humidity conditions. Several days after the release of "dusted" *A. swirskii* and *N. cucumeris* females, most *D. citri* had been killed by *B. bassiana***. As these phytoseiid predators exhibit a relatively high tolerance to this pathogen and are attracted to *D. citri*, **this method might represent a new technique for using the *Beauveria* to control this pest insect.**

Tabla 9. Entomopathogenic fungi for control ***Diaphorina citri* (Asian citrus psyllid)**

Type (Pred/Par/Entomo)*	Stage of Host	Results/Finding	CSA*	Source/ Reference and Location
Beauveria bassiana (Bals.) Vuill.	Nymph Adult	At the laboratory level Padulla and Alves (2009) indicate mortalities of up to 72% with strains of *Beauveria bassiana* (Vuill.) In a study conducted in Cuba, Álvarez et al., (1994), found in Mexican lemon trees *Citrus aurantifolia* Swingle and in plants of *Murraya paniculata* L. (Jack), nymphs and adults of *D. citri*, parasitized by *B. bassiana*. In a research project carried out in Sinaloa, Mexico in 2009 and 2010, the entomopathogen mixture (*Metarrizium anisopliae, Beauveria bassiana and Paecilomyces fumosoroseus*) was sprayed at 10 mL / L-1 of water, obtaining 73% and 87% of control of nymphs in 2009 and 2010 (Sandoval et al. (2010)	CA	(Rivero Aragon and Grillo-Ravelo 2000, Yang et al. 2006) cited in Hall, E.G. (2008) Cuba, China Álvarez et al. (1994) cited in Preza Durán (2011 Padulla and Alves (2009) cited in Preza Durán (2011) Sandoval et al (2010) cited in Preza Durán (2011) Mexico
Capnodium citri Mont.	Nymph	Affect nymphs of D. citri from the second to the fifth stage (Aubert, 1987)	CA	(Aubert 1987) cited in Halbert & Manjunath (2004) and Hall, E.G. (2008) Florida
Cephalosphorium lecanii Zimm (*Verticillium lecanii*)	-	-	CA	(Rivero-Aragon and Grillo-Ravelo 2000, Xie et al. 1988) cited in Hall, E.G. (2008) Cuba, China Kondo et al. (2015b)
Cladosporium sp. nr. *oxysporum* Berk. & M.A. Curtis	Nymph	Affect nymphs of D. citri from the second to the fifth stage (Aubert, 1987)	CA	(Aubert 1987) cited in Halbert & Manjunath (2004) and Hall, E.G. (2008) Florida
Cordyceps bassiana (ascomycete)	Nymphs Adults	Was evaluated for control of *Diaphorina citri* Kuwayama, on Mexican lemon orchard. In nymphs shared statistical equality with	CA	Lezama-Gutiérrez et al. (2014) Mexico

		chemical treatment, except in the second, third and ninth application.		
Hirsutella sp. *H. citriformis* Speare *H. thompsonii* Fisher *H.* (fungus)	Nymph and adults	*Hirsutella citriformis* Speare It stands out for the high percentage of insects attacked in the field. Étienne et al., (2001) mention that *H. citriformis* is able to regulate the populations of *D. citri* naturally and that its occurrence is common during periods when the relative humidity is greater than 80%, which is decisive for its growth and sporulation At the laboratory level Padulla and Alves (2009) indicate mortalities of up to 72% with strains of *H. thompsnii*. Reyes et al., (2009) recorded mortality in the field of D. citri of up to 73% caused by *Hirsutella citriformis* Speare. Preza Duran (2011) managed to identify a fungus of the genus *Hirsutella (Hirsutella citriformis*) with a higher incidence in Persian lemon. The results of this study coincide with that cited by Halbert and Manjunath in 2004, who point out that it is common to observe the fungus *Hirsutella citriformis* Speare, controlling psyllids, when the relative humidity was greater than 80%.	CA	Étienne, et al. (2001) (Rivero-Aragon and Grillo-Ravelo 2000, Subandiyah et al. 2000, Étienne et al. 2001) cited in Hall, E.G. (2008) México, Indonesia, Cuba, Guadeloupe islands Halbert & Manjunath (2004) Padulla & Alves (2009) and Reyes et al., (2009) cited in Preza Durán (2011)
Isaria fumosorosea Wize *(= Paecilomyces fumosoroseus)*	Nymph	At the laboratory level Padulla and Alves (2009) indicate mortalities of up to 72% with strains of *Isaria fumosorosea* Wize. In a research project carried out in Sinaloa, Mexico in 2009 and 2010, the entomopathogen mixture (*Metarrizium anisopliae, Beauveria bassiana and Paecilomyces fumosoroseus*) was sprayed at 10 mL / L-1 of water, obtaining 73% and 87% of control of the nymphs in 2009 and 2010 (Sandoval et al., 2010)	CA	(Subandiyah et al. 2000) cited in Hall, E.G. (2008) Padulla and Alves (2009) cited in Preza Durán (2011) Sandoval et al (2010) cited in Preza Durán (2011) Mexico Indonesia México, Cuba
Lecanicillium lecanii R. Zare & W Gams	Nymphs	-	CA	Xie et al. (1988) Subandiyah et al., (2000) Meyer et al. (2008) Hunter et al. (2011)

				Indonesia México, Cuba, China
Metarhizium anisopliae (ascomycete)	Nymphs Adults	Was evaluated for control of *Diaphorina citri*, on Mexican lemon orchard. In nymphs statistical equality with chemical treatment, except in the second, third and ninth application.	CA	Lezama-Gutiérrez et al. (2014) Mexico
Paecilomyces sp.	Adults	In a study conducted in Cuba, Álvarez et al., (1994), found in Mexican lemon trees *Citrus aurantifolia* Swingle and in plants of *Murraya paniculata* L. (Jack), nymphs and adults of *D. citri*, parasitized by *Paecilomyces* sp.	CA	Álvarez et al. (1994) cited in Preza Durán (2011) Cuba

***Type of NE:** Predator (Pred), Parasite (Par), Entomophatogenic (Entomo)
***Current state of application of the natural enemy (CSA):** Research (Re), Cited application (CA), Biological control demonstrated with results in the field (BCD)

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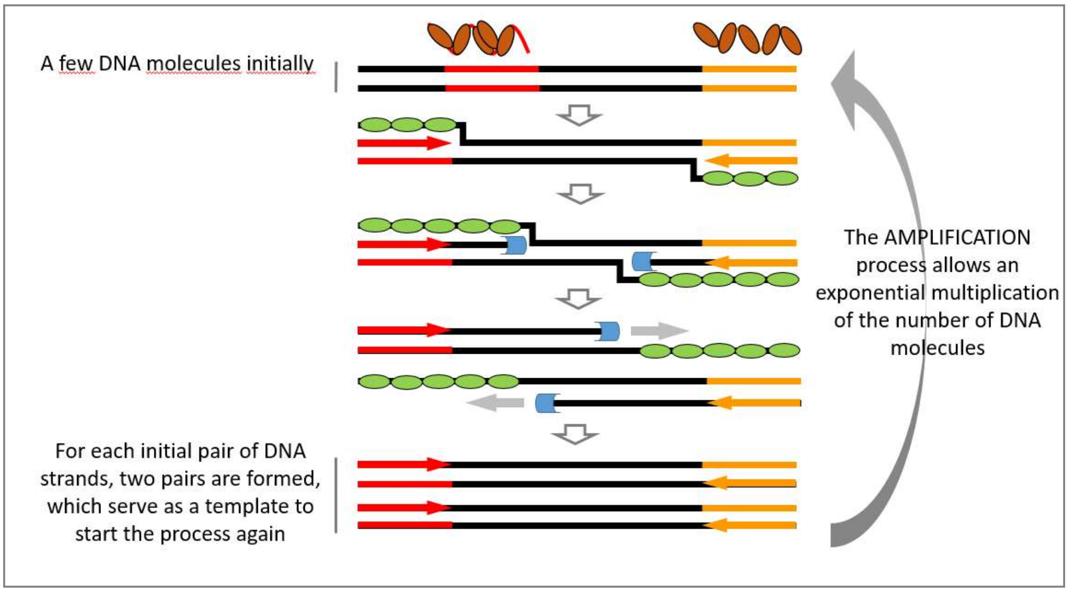
7. Development of HLB identification kit

Objective if Action C.2

To develop a diagnostic protocol for HLB, with a first part containing a kit for on-site detection, together with a simple instruction manual, and a second part for laboratory analysis.

Basis of the techniques

The protocol is based on amplification techniques, in which a few molecules of the target DNA (from bacteria of the genus '*Candidatus* Liberibacter' spp. associated with HLB) are used to achieve an exponential increase in their number. And by means of a fluorescent signalling system they can be detected in real time, as the amplification takes place.



Part One of the Protocol. On-site diagnostic detection kit

Real-time Recombinase Polymerase Amplification Technique (real time RPA)

The advantages of this technique over real time PCR are:

- amplification occurs at temperatures below 40°C, so the equipment required is not as expensive as that for a PCR protocol
- the reaction is very fast, and a detection response can be obtained within 20 minutes
- no DNA extraction and purification is required

This makes it ideal as an on-site detection technique.

Part Two of the Protocol. Laboratory analysis

Real-time Polymerase Chain Reaction (Real-time PCR), in duplex format

The advantages of this technique over the previous one (RPA) are:
- the sensitivity is higher
- allows the determination of the species detected

It is the suitable technique for confirmatory analysis in laboratory.

Format of the on-site detection device

Equipment: portable fluorometer, small size, which can be taken to the field.
Consumables: small tubes with a mixture of reagents, available for use with plant extracts prepared on site in a simple manner

Protocol for using the detection kit
The following figure summarizes the procedure for the analysis of samples in the field.