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in:
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Biological Control of *Aleurocanthus spiniferus* (Quaintance,1903)(Rhynchota-Aleyrodidae)

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Biological Control of *Aleurocanthus spiniferus* (Quaintance, 1903) (Rhynchota-Aleyrodidae)

A Thesis presented by
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Date,

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Abstract

Biological Control of *Aleurocanthus spiniferus* (Quaintance, 1903) (Rhynchota-Aleyrodidae)

The spread of the orange spiny whitefly (OSW), *Aleurocanthus spiniferus* in Italy, with the recent introduction in Balkan countries, is a new challenge that may threat the European citrus agricultural production. *A. spiniferus* has been listed as a quarantine pest to Europe (the EPPO A2 list). It's a polyphagous insect, reported to infest 90 plant species of 38 plant families and it is as one of the most destructive Citrus whiteflies worldwide. In Italy, *A. spiniferus* diffusion from its primary detection sites has been monitored, and up to the end of 2012, the pest was found only in Lecce district; but more recently (till 2015), it has been found in both Taranto and Bari districts. These findings alert about a possible further northern spread of *A. spiniferus*. Classical Biological Control (CBC) is forbidden in Europe, so the chances to use, the local natural enemies among the augmentative biological control programs were discussed on the basis of trials to find out candidates effective indigenous natural enemies. A survey of the most common and abundant natural enemies associated with *A. spiniferus* that was carried out in Apulia region, Italy from April to October 2013- 2014 until June 2015, revealed the presence of 5 species of predators in the area; 4 coccinellids; *Clitostethus arcuatus* (Rossi), *Cryptolaemus montrouzieri*, *Scymnus* sp., and *Oenopia onglobata* (L.), and one chrysopid, *Chrysoperla carnea* (Steph.). In the present study the effect of artificial diets for mass-rearing of some predators was tested. Among 12 artificial diets prepared, only 5 succeeded. *C. arcuatus* and *O. conglobate* artificial diets showed very little performance and seemed poor in the nutrient balance. Rearing of the polyphagous predator, *Harmonia axyridis* on a modified artificial diet showed a success, as it could complete its development on it. Efficacy of the predator, *Macrolophus pygmaeus* against *A. spiniferus* eggs in the laboratory in 2 different temperatures were studied. The adult consumption was low only at the lower temperature. Although the stage and interaction were significant. Therefore, the biocontrol of OSW in non-economic areas is the most promising action to suppress the pest pressure in citrus orchards, as well implementing an integrated pest management approach, involving suitable combinations, are also recommended.

Key words: Apulia, *Aleurocanthus spiniferus*, predators**,** invasive insect, Italy.

CHAPTER 1

1. Introduction

Italy is at high risk of introduction of exotic insects, because of its climatic conditions, which attract many subtropical species, and its position in the middle of the Mediterranean Sea, which makes it a commercial and tourist crossroad (Kenis *et al.,* 2009, DAISIE. 2009). There has been an increasing number of alien insects arriving in Italy since the middle of the last century (57.7%), out of them is Hemiptera, (97%), mostly Sternorrhyncha, that have been introduced mainly through the movements of ornamental plants (Lupi *et al.,* 2014). In Italy, an introductory rate of 98 species per decade was estimated of alien insects that belonged to 10 orders, mostly Coleoptera (38%), Hemiptera (Sternorrhyncha and Auchenorrhyncha) (23%), and Hymenoptera (13%). Most species came from the Nearctic region (26%) and were both phytophagous (63%) and amphigonic (80%) (Inghilesi *et al.,* 2013).

The citrus spiny whitefly, or Orange Spiny Whitefly (OSW), *Aleurocanthus spiniferus* (Quaintance) (Hemiptera: Aleyrodidae), is a pest of citrus (*Citrus* spp.; Rutaceae). In Europe, it was reported for the first time in Italy in 2008 (Porcelli, 2008 and Nutricato *et al.,* 2009). In May 2012, *A. spiniferus* was first found in Croatia, on ornamental potted orange seedlings (*Citrus aurantium* L.) in a nursery garden in Split (Šimala, Masten Milek, 2013). In October 2013, it was observed for the first time in Montenegro (Radonjić *et al.,* 2014). OSW is a polyphagous insect, reported to infest 90 plant species of 38 plant families (Cioffi *et al.,* 2013). The pest affects host plants by sucking on plant sap, but the whiteflies also cause indirect damage by producing honeydew, which promotes sooty mold. Furthermore, *A. spiniferus* was listed as an EPPO quarantine species and has recently been moved from the A1 to the A2 list (Anonymous, 2011). Chemical control has proved to be not effective yet against OSW (Gyeltshen *et al.,* 2010). The pest seems to be well controlled by natural enemies such as hymenopteran parasitoids in its native countries. Most of these species are small wasps belonging to the family Aphelinidae: *Ablerus connectans* Silvestri, *Encarsia divergens* (Silvestri), *E. ishii* (Silvestri), *E. merceti* modesta Silvestri, *E. Nipponica* Silvestri, *E. opulenta* (Silvestri), *E. smithi* (Silvestri), *Eretmocerus serius* Silvestri, *E. silvestrii* Gerling and have proved to be effective in several regions of the world (Silvestri, 1928; Clausen, 1934; Smith, 1945; Quezada, 1974 and Evans, 2008).in Apulia Only larvae of Clitostethus arcuatus (Rossi) (Coleoptera Coccinellidae) were observed preying eggs and 1^{st} and 2^{nd} instar nymphs of A. *spiniferus* on infested plants (Cioffi et al., 2013).

1.2. Study Objectives

The study was proposed to:

1- Monitoring the spread and seasonal abundance of *A. spiniferus* (as invasive insect pest) in Puglia in citrus orchards as well on other host plants.
2- Surveying its natural enemies in the region.
3- Studying the potential of the bio agents found associated with *A. spiniferus* under lab. conditions.
4- Mass-rearing of the promising bio agents and trails to release them to suppress the pest population.
5- Coming out with recommendations to control the pest.

CHAPTER 2

2. Literature review

2.1. Italian Citrus Fruit Outlook

Citrus is largely cultivated in southern Italy (Sicily, Calabria, Apulia, Basilicata, Sardinia, Campania) and to a minor extent in some areas of the few central (Tuscany and Latium) and northern (Liguria). The citrus agro-ecosystem is certainly one of the most complexes with a very rich harmful arthropod fauna and with a vast number of natural enemies; parasitoids, predators and entomopathogens. A total of 143 pest problems was reported, including 108 insects, 10 mites, 1 nematode, 14 fungi, 2 bacteria, and 8 viruses. The insects include 2 Orthoptera, 66 Hemiptera, 8 Thysanoptera, 13 Coleoptera, 12 Lepidoptera, 1 Diptera, and 6 Hymenoptera. From the reported pest problems, 46 were considered key-pests, namely; 26 insects, 4 mites, 1 nematode, 1 bacterium, 9 fungi, and 5 viruses and virus like diseases. Major key-pests, *i.e.,* reported as key-pests in at least (50%) of the counties, include the Medfly, *Ceratitis capitata* (91% of the counties), the citrus red scale, *Aonidiella aurantii* (73%), the citrus leafminer, *Phyllocnistis citrella* (64%), and the citrus mealybug, *Planococcus citri* (64%) and the citrus flower moth, *Prays citri* (45%) (Zappalà *et al.,* 2010, and Greco, 2015).

2.2. Invasive insects in Italy

More than 500 insect pests in the Italian ecosystems are considered exotic. About 200 of these species have been introduced and established in Italy since 1970 (Disie, 2009). The intensification of the plant trades between Italy and geographically distant commercial partners is one of the main sources of these introductions. Climatic abnormalities have also favoured the spread and acclimation of these exotic organisms (Santi, 2015). The initial success of an exotic pest is strictly related to habitat characteristics, its biological performance, such as fecundity, dispersal ability and interactions with other species (Gröbler and Lewis, 2008, and Costanza and Lupi, 2011). Roques et al. (2009) reported 1306 alien insect species (AINS) in Europe, and showed that their rates of invasion differed among European countries. According to Zapparoli (2008), Italy hosts a total of 728 AINS, which makes the Italian alien entomo-fauna the richest in Europe. Costanza and Lupi (2011) listed a total of 291 exotic insects. Introduced the new species (115) are ordered in chronological lists according to their main host plants (Pellizzari, 1997). They are mainly pests of ornamentals, woody plants and citrus. The three orders, accounting for the most of the introductions are Homoptera (76% of the total number of introduced species), Coleoptera (10%), and Lepidoptera (14%). The majority of the introduced species have come from America (36%), Asia (25%), Africa (17%), Australia (7%) (Table 1). In some cases, Italy has been the first noticed, focus of an exotic pest in Europe. From there the pest has expanded throughout Italy and towards neighboring countries.

2.3. The orange spiny whitefly *Aleurocanthus spiniferus* (Quaintance,1903)

The orange spiny whitefly (OSW), *Aleurocanthus spiniferus* (Quaintance) (Rhynchota-Aleyrodidae), was recorded by Kuwana (1928) as the most destructive aleyrodid species attacking citrus in tropical Asia. Experts considered it as the most important pest among 4 or more species of white flies attacking citrus in Japan. It was rated by Clausen (1934) as the 7th most important citrus insect species in Japan. A heavy outbreak of citrus in the Kyushu area of that country caused great damage to trees and loss of fruits until it was brought under control in the 1920s by the parasitic wasp, *Prospaltella smithi* Silvestri. Clausen (1934) who reported *Amitus hesperidumvariipes* Silvestri, as another gregarious parasitoid of *A. spiniferus* in tropical Asia. It oviposits in the young larvae immediately after hatching and before they become fixed to the leaf. More recently, *A. spiniferus* became the most serious pest of citrus on the island of Guam until brought under control by the introduction of parasites in 1952 . In 1974, *A. spiniferus* was discovered in Hawaii in the island of 0ahu, where it rapidly became a principal pest. Heavy infestations of OSW caused a rapid deterioration of the trees and crop failure to all citrus varieties and had led to trees' mortality.

Table 1: List of invasive exotic insects in Italy in the last decade (Costanza, 2011)

Species	Order/family	Common name	Host	Origin	Year of introduction
ORNAMENTALS					
Cacyreus marshalli	Lepidoptera -Lycenidae	Geranium bronze butterfly	*Pelargonium* and *Geranium*	South Africa	1996
Anoplophora chinensis	Coleoptera -Cerambicidae	Citrus Longhorned Beetle	ORNAMENTALS	Asia	2001
Paysandisia archon	Lepidoptera -Castniide	American palm borer	Aceraceae (*Phoenix canariensis*, *Trachycarpus* sp., *Chamaerops humilis*,)	South America (Argentina, Brazil, Paraguay, Uruguay)	2002
Rhynchophorus ferrugineus	Coleoptera- Curculionidae	Red palm weevil	Aceraceae (*Phoenix canariensis, Phoenix dacylifera,, Washingtonia robusta, Cocos nucifera*…)	Asia	2005
Psacothea hilaris	Coleoptera -Cerambycidae	Yellow-spotted Longicorn Beetle)	Moraceae (*Ficus* spp., *Morus* spp.)	Japan, China, Taiwan	2005
Anoplophora glabripennis	Coleoptera -Cerambycidae	Asian Longhorned Beetle	ORNAMENTALS	Asia	2007 -2009
CROPS					
Diabrotica virgifera virgifera	Coleoptera -Crisomelidae	Western Corn Rootworm	Maize (*Zea mays*)	Central America	1998
Dryocosmus kuriphilus	Hymenoptera -Cynipidae	(Oriental chestnut gall wasp)	*Castanea* spp. and their hybrids.	China	2002
Lissorhoptrus oryzophilus	Coleoptera- Eririnidae	Rice Water Weevil	Rice (*Oryza sativa*). Wild grasses (Poaceae and Cyperaceae) as alternative hosts.	America	2005
Tuta absoluta* *(Meyrik)	Lepidoptera- Gelechiidae	American Tomato Pinworm	Tomato (*Solanum lycopersicon)*. Potato (*Solanum tuberosum*) and other Solanaceaea are also reported as a host.	South America	2008
Drosophila suzukii	Diptera -Drosophilidae	Spotted Wing Drosophila	Fruit crops: small fruit crops (strawberries, raspberries, blackberries, blueberries) fruit trees (peaches, plums, apples) and grapevine.	Asia (China, India, Japan. Thailand, Korea)	2009
POLYPHAGOUS					
, Aleurocanthus spiniferus* *(Quaintance,1903)	Rhynchota - Aleyrodidae	The orange spiny whitefly	Polyphagous	Asia (China, Japan)	2008

2.3.1. Host plants

A. spiniferus is a polyphagous pest. *Citrus* spp. Are the main hosts of economic importance, but *A. spiniferus* has been recorded on other crops and ornamental plant species for example: Rutaceae, Vitaceae, Araliaceae, Ebenaceae, Leguminosae, Caesalpiniaceae, Malvaceae, Lauraceae, Moraceae, Punicaceae and Rosaceae, here reported to infest 90 plant species of 38 plant families (Kanmiya *et al.,* 2011; Cioffi *et al.,* 2013, and Kenawy *et al.,*2014)

2.3.2. Geographical distribution

A. spiniferus originated in southeast Asia and has spread widely in tropical and subtropical Asia, and into Africa and the Pacific (Fig. 1) (Cioffi *et al.,* 2013 and EPPO, 2015).

Asia: Bangladesh (Alam *et al.,* 1965), Bhutan, Brunei Darussalam, Cambodia, China, Hong Kong, India (Singh, 1931), Indonesia (Fletcher, 1919), Japan,Korea Democratic People's Republic, Korea Republic, Lao, Macau, Malaysia (Gater, 1924), Pakistan (Gentry, 1965), Philippines (Peterson, 1955), Sri Lanka, Taiwan, Thailand, Viet Nam.

Africa: Kenya, Mauritius (Moutia, 1955), Nigeria, South Africa (van den Berg *et al.,* 1990), Swaziland, Tanzania (including Zanzibar), Uganda. (Newstead, 1911)

North America: USA (Hawaii only).

Oceania: Australia, Guam, Micronesia, Northern Mariana Islands, Papua New Guinea.

EU: Italy (Porcelli, 2008), Coratia (Šimala, 2013), Montenegro (Radonjić *et al.,* 2014).

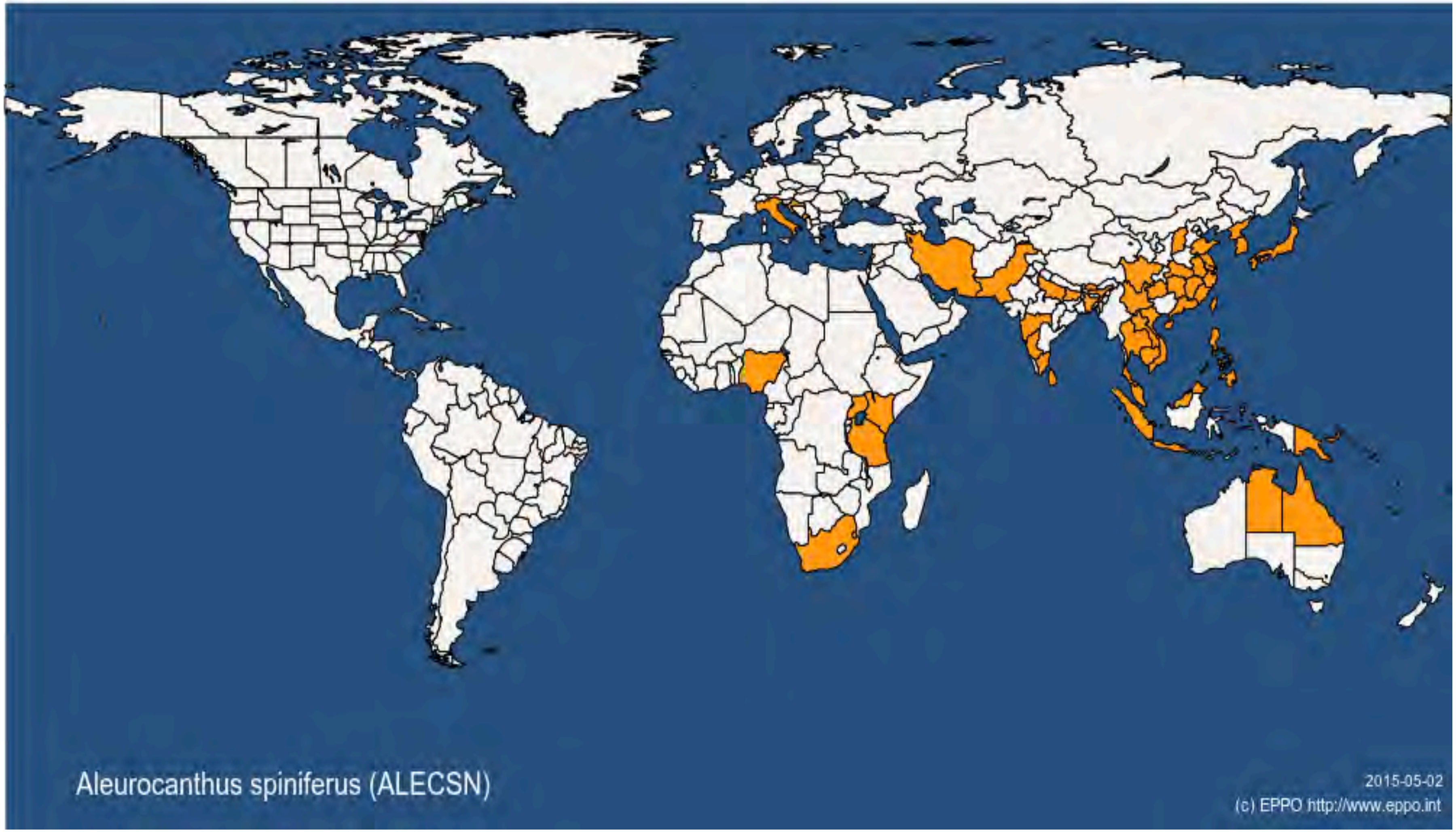


Figure 11: Geographical distribution of *A. spiniferus* (last update 10/07/2015)

2.3.3. Biology

Many aspects of *A. spiniferus*, including the biology, behaviour and ecology have been thoroughly investigated, all stages of *A. spiniferus* may be found throughout the year. The biology of OSW (EPPO/CABI, 1996) is as follows: Eggs, laid in a spiral path on leaf undersides in batches of 35-50, hatch in 4 to 12 days depending on conditions. Active, black, flattened six-legged crawlers (nymphs) emerge. There were 6 generations of OSW and overwintering took place at the nymphal stage in November-March. The survival rate from egg to adult in each generation averaged < 20%. Adult females of the 1^{st} and 2^{nd} generations preferred to oviposit on the lower and middle sections of the trees rather than on the upper one. Adults of the 6^{th} generation had no preference for oviposition location. Fourth-instar nymphs was most abundant in the central and least abundant in the upper section of the trees (Kanmiya *et al.,* 2011). The crawlers then insert their mouthparts into the leaves and begin sucking phloem sap. They then moult, losing their legs in the process, to become minute, flattened, oval bodies attached to the leaf by their mouthparts. After two more moults, the adults emerge. Both sexes are winged and feed on sucking phloem sap. Each female may lay 35-100 or more eggs in her lifetime (Chien and Chiu, 1986, and Chen *et al.,* 2016). Depending on the conditions, the life cycle generally lasts 2-4 months. Development is most favoured at temperatures of 20-34°C (optimum 25.6°C) and relative humidities of 70-80%. The species does not survive at temperatures below freezing and is not found in areas with temperatures of 43°C or over.

2.3.4. Symptoms and damage

Dense colonies of immature stages develop on leaf undersides; the adults fly actively when disturbed. Leaves and fruits have spots of sticky, transparent honeydew, which become covered in black sooty mould fungus (Fig. 2). A heavy infestation gives trees an almost completely black appearance. Heavy infestations reduced the vigor and fruit yield of plants. Serious infestation causes a general weakening due to sap loss and the growth of sooty mold (Anonymous, 1974). The under surfaces of infested leaves were heavily infested by OSW and the upper surfaces were covered with black sooty mold (Muniappan *et al.,* 1992). Infested citrus trees have numerous small, brownish to black scales with a short fringe of white wax on the underside of many leaves (Marutani and Muniappan, 1991). *A. Spiniferus* excretes copious amounts of sugary honeydew, which coats the leaf and fruit surfaces. The sooty mold fungus develops on the honeydew, reducing respiration and photosynthesis and rendering plants and fruits unsightly and unsaleable. Badly contaminated foliage may drop.

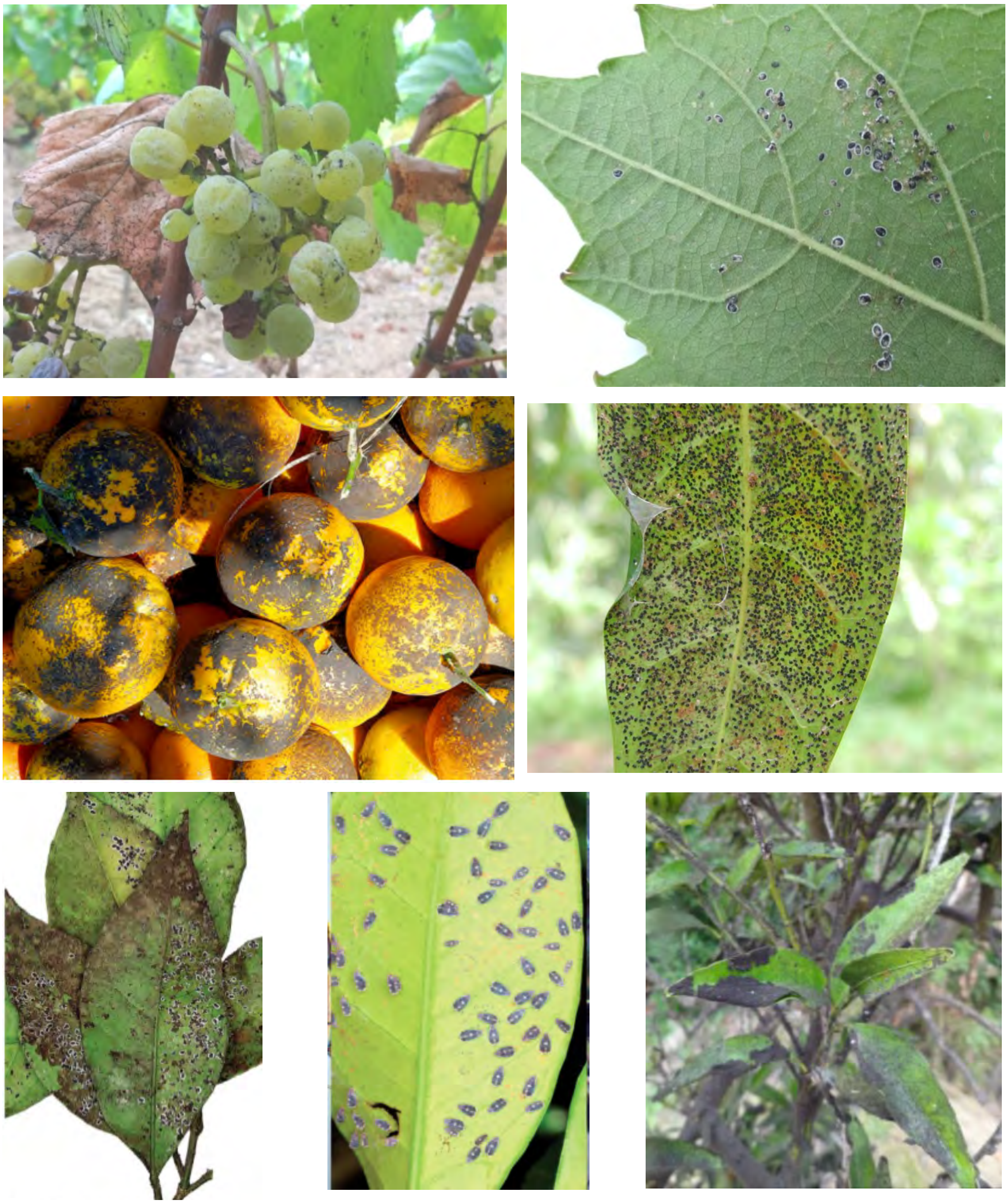


Image Credit: Francesco Porcelli, DiBCA sez Entomologia e Zoologia, University of Bari, EPPO

Figure 2: Damage caused by *A. spiniferus*

2.3.5. Diagnostic characteristics of *A. spiniferus* (EPPO,2008)

- **Eggs**

Elongate-oval to kidney-shaped, 0.2 mm long (0.01 in.), laid in a very characteristic spiral pattern, attached to the underside of leaves by a short pedicel; yellowish 1-2 days after laying, turning darker to brown and black as the embryo develops (Fig. 3). Incubation varies depending on temperature and averages 7-22 days.

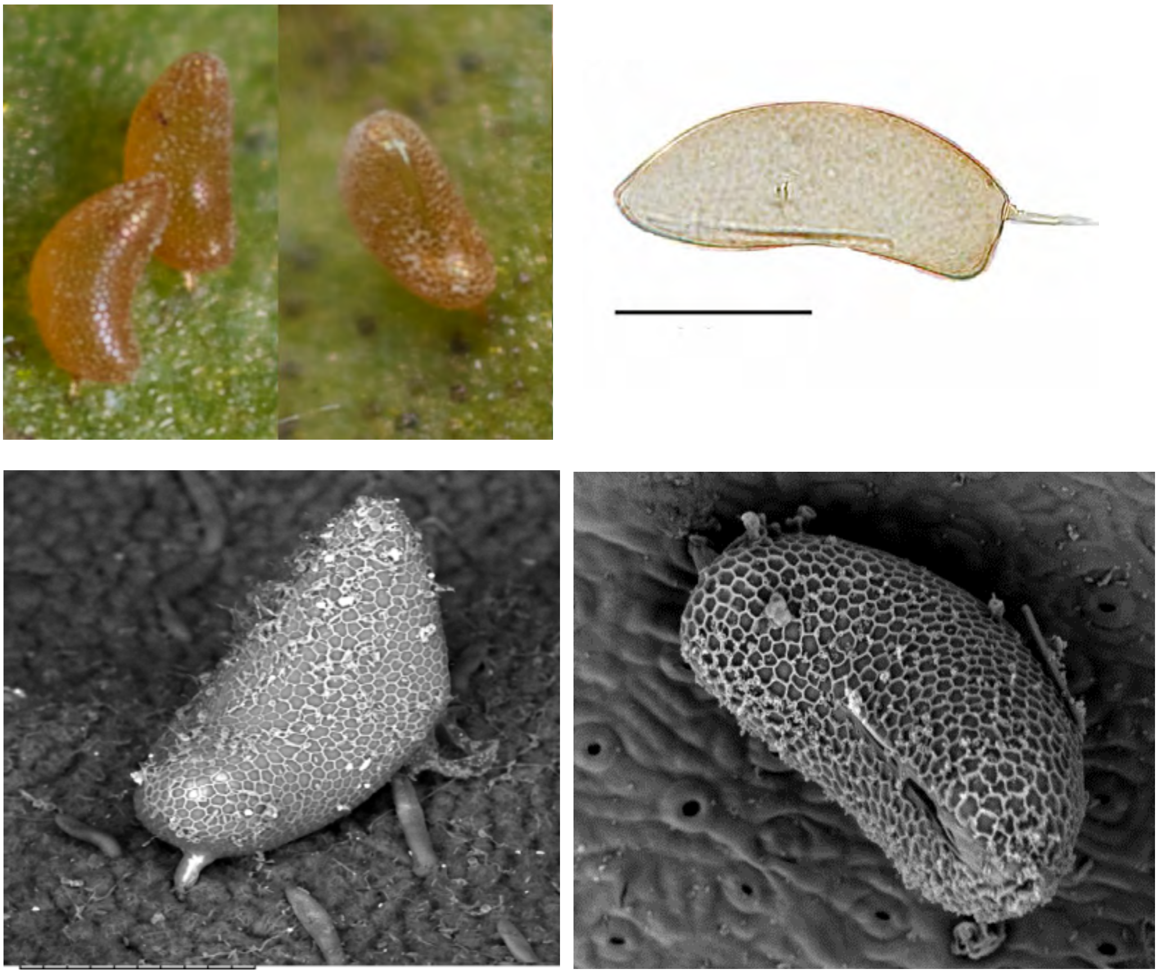


Image Credit: Francesco Porcelli, Elkenawy Ahmed, Disspa sez Entomologia, University of Bari, Kanmiya 2011

Figure 3: Edunculate egg attached to leaf host

❖ Immature stages

- **1st instar**: 6-legged, elongate, 0.3 x 0.15 mm, dusky in colour, with 2 long and several shorter, radiating spiny filaments.
- **2nd instar**: No legs, ovate-convex, 0.4 x 0.2 mm, dark-brown to pale-black with yellow markings, with easily distinguished, radiating spiny filaments.
- **3rd instar**: More ovate, 0.74-0.87 mm, generally black with a rounded, greenish spot on the anterior part of the abdomen, spiny filaments obvious.

Image Credit: http://wireway25.rssing.com/chan-13756564/all_p41.html

Figure 4: Juveniles immature stages on underside a citrus leaf

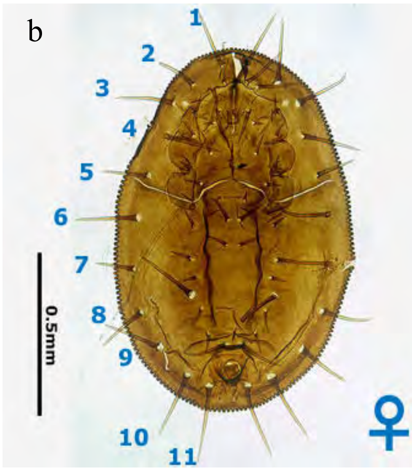
- **4th instar = "puparium":** Ovate, shiny-black, females about 1.25 mm in diameter, males slightly smaller, up to 1 mm in diameter. Dorsal surface with many long, acute glandular spines; insect surrounded by a white fringe of waxy secretion. Exuviae of earlier instars often remain stacked up on the median area of an immature insect.

Figure 5: a) *A. spiniferus* 4th instar "puparium"

b) 4th instar carrying a molting shell of 2-3 instar

Photo © Ochatatemushi,2013

Male pupa is about 1.23mm long by 0.88 mm wide, 10 submarginal spines



Female pupa is about 1.23mm long by 0.88 mm wide, 11 submarginal spines

Figure 6: *Aleurocanthus spiniferus*: a),b) general aspect of the mounted puparium Male and female; c) marginal teeth using light microscope with phase contrast; d) posterior end of the puparium.

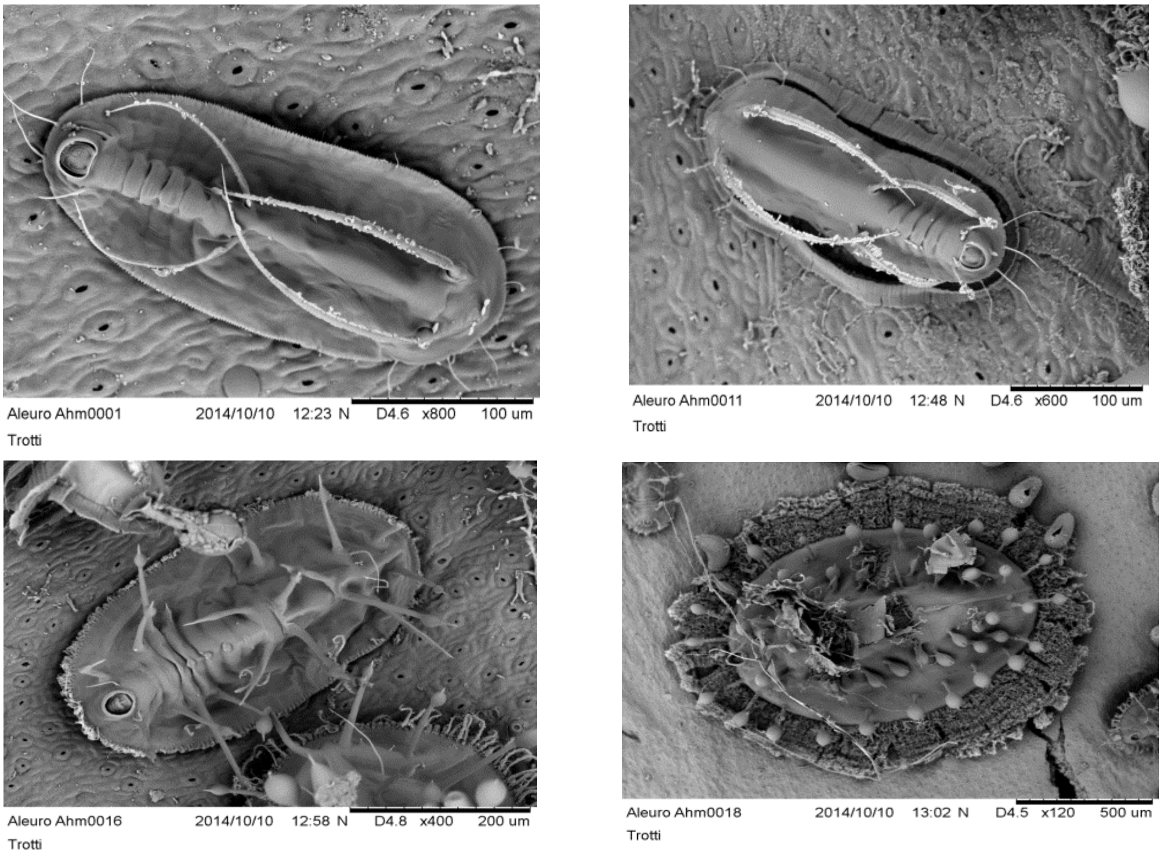


Image Credit: Elkenawy Ahmed, Disspa sez Entomologia e Zoologia, University of Bari

Figure 7: Differant *A. spiniferus* immature stages (SEM)

❖ Adult

1.3 - 1.6 mm (0.05 - 0.063 in.) in length. Clear spots across the forewing; appear to create a white band pattern. Adults emerging from pupal cases are red with light-coloured legs and reddish eyes. The body colour is bright red at emergence, but changes to a dusty slate-gray color within 24 hours. The front of the head is pale yellow.

Image Credit: Elkenawy Ahmed, Disspa sez Entomologia e Zoologia, University of Bari, Ochatatemushi,2013

Figure 8: Adults of *A. spiniferus*

Means of movement

Adults of *A. Spiniferus* are capable of limited down-wind flight, but this is not a major means of long-range dispersal (Meyerdink *et al.,* 1979). Species of *Aleurocanthus* have been intercepted on the leaves of infested host plants moving in international trade (*e.g.* USDA, 1988).

2.4. Pest Management

Management of heavy whitefly infestations is very difficult. White flies are not well controlled by any available insecticides. The best strategy is to prevent problems from developing in the orchard to the extent possible. The concept that pest control should be based on economic as well as ecological considerations has been a pervasive force in integrated pest management over the past 30 years (Peterson,1955). In many situations, natural enemies provide adequate control of white flies; outbreaks may occur, if the natural enemies that provide biological control of white flies are disrupted by insecticide applications, dusty conditions, or interference by ants. In small size orchards, whitefly population in the early stages of population development can be held down by a vigilant program of removing infested leaves, vacuuming adults, or hosing down (syringing) with water sprays. Hand-removal of heavily infested leaves with the nonmobile nymphal and pupal stages may reduce populations to levels that natural enemies can contain. Water sprays (syringing) may also be useful in dislodging adults. A small, handheld, battery-operated vacuum cleaner has also been recommended for vacuuming adults off leaves (Fassihi *et al.*, 2014). Vacuum can be done in the early morning or other times when it is cool and white flies are sluggish.

- **Cultural control**

3. Prune infested stems, branches and fruits and burn them.
4. Apply mulch, manure or synthetic fertilizers to assist plant vigour.
5. Destroy ant nests with boiling water, without damaging the plants infested with the scale insect; without the
6. ants, parasitoids will bring about natural control of the scale insect.
7. For trees, prune low branches and remove weeds to stop ants reaching leaves and fruits.

- **Chemical control**

In general, chemical control has not been effective against the orange spiny whitefly. However, whiteflies can be controlled using horticultural oil (made from petroleum), white oil (made from vegetable oils), or soap solution .

- White oil:
  - 3 tablespoons (1/3 cup) cooking oil in 4 litres water. /
  - ½ teaspoon detergent soap.
  - Shake well and use.
- Soap:
  - Use soap (pure soap, not detergent).
  - 5 tablespoons ofsoap in 4 litres water, OR
  - 2 tablespoons of dish washing liquid in 4 litres water.

Commercial horticultural oil can also be used.White oil, soap and horticultural oilsprays work by blocking the breathing holes of insects causing suffocation and death. Spraythe undersides of leaves; the oils must contact the insects. A second application ofsoap or oils may be necessary after 3-4 weeks. Gyeltshen J, Hodges A, Hodges GS (2010)

Use synthetic pyrethroid insecticides to kill ants if they are present, attracted to the honeydew. Generally,insecticides are not effective against orange spiny whitefly as they are likelyto destroy natural enemies which otherwise will bring about satisfactorycontrol.

2.5.1. Traps

Yellow sticky traps can be posted around the garden (orchard) to trap adults. Such traps won't eliminate damaging populations, but may reduce them somewhat as a component of an integrated management program relying on multiple tactics (Shen and Ren 2003 and Gu *et al.,* 2008). White flies do not fly very far, so many traps may be needed. Although commercially available sticky substrates such as Stickem or Tanglefoot are commonly used as coatings for the traps,

2.5.2.Biological control of *A. spiniferus*

It was believed that the pest originated in south China, in the native range of citrus and where the spiny black fly was known to occur. Entomologists and horticulturalists planned to search for natural enemies in China. Silvestri of Portici, Italy, was in a temporary foreign exploration trip for the University of California to find parasitoids for the California red scale, visited Japan. Biological control possibilities were considered in Japan in the early 1920's. *Encarsia smithi*, and the coccinellid *Cryptognatha* sp. were imported from China (Kuwana & Ishii, 1927). Only the parasitoid had established, and it quickly gave complete biological control (DeBach, 1974). The whitefly first appeared in Guam (an unincorporated and organized territory of the United States) in 1951. In 1952, *Encarsia smithi*, *E. clypealis*, and *E.opulenta* (Silvestri), *Eretomocerus serius* and *Amitus hesperidum* were imported from Mexico (Peterson, 1955) for a classical biological control program. Only *E. smithi* and *A. hesperidum* established, but complete biological control was attained primarily through the action of *E. smithi*. In Hawaii, the whitefly was first found in 1974, and *A. hesperidum* was introduced from Mexico and *E. smithi* from Japan. Both parasites established, but the control status was not reported (Nakao and Funasaki, 1975). However, in June 1926 parasitoid activity was observed on the release trees, and 74% of the whitefly pupae showed exit holes from which adult parasitoids had emerged. They spread rapidly and were aided by distribution of leaves bearing parasitized pupae, so that within a short time, the pest was almost completely eliminated (Kuwana, 1928). DeBach (1974) maintained a perfect control that remained to the 1970's. In various countries, the efficacy of biological control agents has been studied against several species of citrus-feeding white flies, mainly by means of predator and parasitoid insect species, and secondary by the use of entomopathogenous fungi. In addition to it, the considerable predacious activity of *Clitostethus arcuatus* (Rossi) against the citrus whitefly was reported . Coffi *et al.,* 2011 discussed the Augmentative Biological Control (ABC) chances, on the basis of trials to discover a candidate effective indigenous natural enemies in Apulia-Italy to control OSW.

Several species of beneficial organisms are efficient for biological control of whiteflies.

2.5.2.1. The bright green mirid bug *Macrolophus pygmaeus* (Rambur)

Macrolophus pygmaeus (Heteroptera- Miridea) is a polyphagous predator originally from the Mediterranean. Both larvae and the adults are predators feeding on several soft-bodied pests such as; aphids, mites, thrips and moth eggs (Albajes and Alomar, 2002, and Perdikis and Lykouressis, 2004). Although, the predator preys on the whitefly, *Trialeurodes vaporariorum* [Homoptera: Aleyrodidae] (BIOBEST, 2012). An adult can prey on an average of 6 whitefly larvae in a day and suck empty about 40 to 50 eggs (Rasdi *et al.,* 2009). *M. pygmaeus* had the shortest period of nymphal development on eggplant with *T. vaporariorum*, followed by *M. persicae*, *M. euphorbiae*, *A. gossypii*, and *Tetranychus urticae* Koch. Mortality of *M. Pygmaeus* nymphs were real Bee pollen and pollen from *E. elaterium*, when offered separately, were sufficient to support successful predator nymphal development and survival (Perdikis and Lykouressis, 2000). Bee pollen contributed considerably to the development and survival of the nymphs when it was included in diets containing other food sources, like eggplant leaves and *M. persicae*. *M. pygmaeus* could survive in the absence of prey and complete its development on tomato, eggplant, cucumber, pepper, and green beans (Perdikis and Lykouressis 2000 and De Backer *et al.,* 2015). This is of particular importance for its establishment and effectiveness at the beginning of the growing season when the temperature is relatively low and the prey is absent or scarce, as well as in summer when temperatures are high enough (around 30°C or even higher)

2.5.2.2. *Clitostethus arcuatus* (Rossi) (Coleoptera: Coccinellidae)

Clitostettius arcuatus (Rossi), a predator of aleyrodids and other insects and mites. It is one of several coccinellid predators feeding on white flies (Gerling, 1990). It is widely distributed in the Mediterranean and surrounding regions. It was also reported from the former USSR (Agekyan, 1977), Sardinia (Delrio *et al.,* 1979, and Ortus and Ibba 1985), Portugal (Magalhaes, 1980), Africa (Fursch, 1987), Italy (Gargani, 1990), the USA (Bellows *et al.,* 1990a; 1992b), Germany (Ziegler, 1993), former Yugoslavia (Peric *et al.,* 1997), Greece (Katsoyannos *et al.,* 1998) and Iraq (Al-Alaf *et al.,* 2001). In Iran, *C. arcuatus* was reported for the first time by Yazdani and Assadi (1989) in Fars Province and since then has been observed in other parts of the country (Lotfalizadeh, 2001 and Tavadjoh, 2005) *C. arcuatus* is a specialist whitefly predator, easily identified due to the pale horseshoe-shaped marking on the light- to dark-brown elytra. It is a native throughout the Western Palaeartic, particularly around the Mediterranean, but not in Scandinavia or the Baltic (Booth

& Polaszek, 1996). The predator prefers aleyrodid species (Mills, 1981) and has been used to control *S. phillyreae* in California. It preys on other species of Homoptera including *T. vaporariorum*, *B. tabaci* (Gerling *et al.,* 2001), *D. citri* (Agekyan, 1977, and Basiri *et al.,* 2004), *Aleurotrahelus jelinekii* (Mills, 1981), and *Aleurodes proletella* (Bathon and Pietrzik, 1986). In addition, it also feeds on the eggs of *Tetranychus urticae* (Liotta *et al.,* 2003) as well as aphids (Gerling *et al.,* 2001).

It feeds on whitefly eggs, nymphs and adults with perdition apparently primarily on the eggs and nymphs when all stages are present (Priore, 1969 and Bathon and Pietrizik, 1986). Loi (1978) mentioned that *C. arcuatus* was first described as *Scymnus* by Rossi (1794) and added that wise describe this genus as *Clitostethus* in 1885. Zarabi, (2010) studied *C. arcuatus* biology and predation on the greenhouse whitefly, *T. vaporariorum*. The larvae consumed an average of 992.2±36 eggs during its total larval developmental period, with a daily mean of 45.8±0.5. Females, males and one pair of *C. arcuatus* (♀,♂) consumed an average of 17.2±0.4, 10.6±0.8 and 23.1±0.5 nymph/day; 28.5±0.9, 20.3±0.6 and 47.2±0.6 pupa/day and 8±0.3, 6.5±0.54 and 13.6±0.4 adult/day, respectively. Females laid an average of 3±0.23 eggs/day. The predator was highly correlated with the dominant whitefly *Siphoninus phillyreae* in Egypt . Mesbah, (2008) studied the population dynamics of *C. arcuatus* and *S. Phillyreae* on citrus in Egypt and indicated that the pest and the related predator were relatively dominant in August. The maximum number of *C. arcuatus* ranged from 13 to 44 beetles per 100 leaves (Abd-Rabou, 2010). *C. arcuatus* was positively affected by the perimeter of the host patch, the total area of citrus orchards and the mean proximity of urban vegetation patches (Grilli *et al.*, 2015). Bellows *et al*. (1992) studied the predator's biology on different temperature degrees. At 28.2°C, its development from egg to adult required a mean of 15.6 days, egg-to-adult survival was (78%), sex ratio of surviving progeny was (1:1) and females lived an average of (82 days) and laid an average of (202 eggs). Development rates and fecundity were slightly lower and survival was similar at 21.1°C, while they were reduced substantially at 32.2°C.

Loi, (1978), reported that the predator had 4 generations a year, laying 11-41 eggs each on the lower surface of citrus leaves. Larvae hatched after 3-8 days and pupated after a further 8-24 days. According to the temperature; it fed mainly on the eggs but also on the pupae of *D. citri*. Adults, which emerged 3-8 days after pupation, overwintered from September or October until mid-March in the glass-house or to early April in the open air and fed on the adults of *D. citri.* Arthropods prey on *C. arcuatus* were the chrysopid larvae (which attacked both *Clitostethus* larvae and *D. citri),* and sometimes the spiders of the genus *Salticus* (Attus), which was found only on citrus trees cultivated against walls or in narrow spaces between houses. Agekyan, (1977) stated that the egg, larval and pupal stages were found to last 5, 13-14 and 4-5 days, respectively, at 24-27 ºC, and the whole period from oviposition to adult emergence lasted 23-25 days. The adults that appeared in late May or early June, became numerous on the young shoots of citrus plants infested with adults of the aleyrodid of the overwintered generation, and laid eggs singly among the whitefly colonies Mesbah, A.H., (2001,2000) . When the larvae hatched, they fed on the eggs and young larvae of the latter, this activity lasting throughout the month. Pupation began towards the end of June, and only very few larvae still persisted in mid-July. Bellows *et al. (*1992) reported that *C. Acts* were introduced into infested areas in 1990 and 1991 as a biological control agent to control the aleyrodid *Siphoninus phillyreae*in California. Liotta, (1981) studied the biology and behaviour of *C. arcuatus* in Sicily, Italy and stated that it had 4 generations a year, the 1^{st} from mid-April to mid-May, the 2^{nd} throughout June, the 3^{rd} throughout July and the 4^{th} from mid- or late August to the end of September. The adults overwintered in cracks in the barks or in walls, but came out into the citrus orchards on mild sunny days. Cannibalism was observed among the larvae (ate other larvae of their own species) and adults (ate the eggs of their own species). Adults were occasionally observed to feed on citrus leaves in the summer.

2.5.2.3. The multicolored Asian lady beetle, *Harmonia axyridis* (Pallas),

The multicolored Asian lady beetle, *Harmonia axyridis* (Pallas), is a well-known aphid predator in its native Asian range (*e.g.,* Yasumatsu and Watanabe, 1964; Hukusima and Kamei, 1970, and Hukusima and Ohwaki, 1972,). The presumed native distribution of *H. axyridis* extends from the Altai Mountains in the west to the Pacific Coast in the east, and from southern Siberia in the north to southern

China in the south (*e.g.,* Korschefsky, 1932, Dobzhansky, 1933, Chapin, 1965, Sasaji, 1971, and Kuznetsov, 1997,).

H. axyridis has been released in Europe (Katsoyannos *et al.,* 1997, and Iperti and Bertand, 2001) the mean duration of each stage is as follows: egg 2.8 days, first instar 2.5 days, second instar 1.5 days, third instar 1.8 days, fourth instar 4.4 days, pupa 4.5 days (LaMana and Miller, 1998). Development from egg to adult was shown to require 267.3 degree days above a lower developmental threshold of 11.2° C in the United States (LaMana and Miller, 1998), and 231.3 degree days above a lower developmental threshold of 10.5° C in France (Schanderl et al., 1985). Temperature not only influences the rate of development, but also adult weight. Larvae reared at higher temperatures produce smaller adults than larvae reared at lower temperatures (Kawauchi, 1979). Diet has also been shown to impact larval development. Hukusima and Ohwaki (1972) found that developmental time decreased with an increase in aphid consumption. The species of aphid preyed upon and the species of plant the aphids develop on can effect larval developmental time, adult longevity, and fecundity (Hukusima and Kamei, 1970). Adults typically live 30 to 90 days depending on temperature (El-Sebaey and El-Gantiry, 1999, He et al., 1994, Soares et al., 2001). However, adults may live up to three years (Savoiskaya, 1970a, Savoiskaya, 1970b). Pre-mating and pre-oviposition periods were shown to decrease with increasing temperature (He et al., 1994, Stathas et al., 2001). Under laboratory conditions, females can produce up to 3,819 eggs at a rate of 25.1 eggs per day (Hukusima and Kamei, 1970). However, Stathas et al. (2001) reported a lower maximum total fecundity of 1,642 eggs. Females typically oviposit batches of approximately 20 to 30 eggs (Takahashi, 1987).

H. axyridis has been utilized in augmentative biological control in Asia (e.g., Seo and Youn, 2000), Europe (e.g., Trouve et al., 1997), and North America (e.g., LaRock and Ellington, 1996).Under field conditions, LaRock and Ellington (1996) reported an effective integrated pest management program, incorporating inoculative releases of *H. axyridis* and other predators, for the pecan aphid complex. Mass releases of *H. axyridis* provided effective control of scale insects in pine forests (Wang, 1986). *H. axyridis* was also effective when released against Phorodon humuli on hops (Trouve et al., 1997). The relative ease of rearing *H.*

axyridis makes it particularly attractive for augmentative biological control. Matsuka and Niijima (1985) describe a system for mass rearing *H. axyridis*. It can be reared on a variety of aphid species (Hodek, 1996). Non-aphid diets, such as the eggs of various Lepidoptera (Abdel-Salam and Abdel-Baky, 2001, Schanderl et al., 1988), pulverized drone bee brood (Okada and Matsuka, 1973), eggs of brine shrimp (Hongo and Obayashi, 1997), and various artificial diets (Dong et al., 2001), can also be used. Nutritional analyses of various diets and their impact on fitness parameters of *H. axyridis* have been examined in detail (Matsuka and Takahashi, 1977, Niijima et al., 1986, Specty et al., 2003). One caveat for the use of factitious prey or diets is that foraging efficiency may be reduced, due to changes in search patterns, when *H. axyridis* is released on a target prey (Ettifouri and Ferran, 1993). Rearing conditions other than diet have also been examined for H. axyridis. Fecundity can be increased by prolonging the amount of time females spend with males (Pando et al., 2001). Ongagna and Iperti (1994) reported how temperature and photoperiod can be altered to promote or deter diapause during rearing.

2.5.2.4. *Oenopia conglobate* (L.,1758) (Coleoptera-Coccinellidae)

Oenopia conglobata is a species of Ladybird (Coccinellidae) native to continental Europe, Asia and Africa . The activities run from April to October then beetles overwinter under the bark of trees, mostly poplars, elms, plane, oak and horse chestnut. The species is also found nesting between the panes of double-glazed windows. It has also been reported on whiteflies, particularly of *Bemisia tabaci* (Gang Wu et al., 2011) and *Trialeurodes vaporariorum* (Peric et al., 2009)..Sadeghi 2004 studied The predation activity of larvae and adult of *Oenopia conglobata* (L.) as a predator of aphids and psyllids of forest, fruit and crop developmental periods from egg to adult emergence were 2.2 ± 0.08, 2.4 ±0.03, 2.33 ± 0.03, 2.13 ±0.03, 4.4 ±0.03, 1.6 ± 0.01 and 4.53 ± 0.03 days. Mean of daily oviposition and total of egg laying female adult were 25.24 ±0.79 and 1435 ± 51.2 numbers. Mokhtari 2014 The results showed that 25 to 30 °C are suitable temperatures for rearing this predator. Mean of daily feeding for 1-4 larvae instars and female adults were 9.09 ± 0.38, 22.87 ± 0.44, 37.57 ± 0.75, 52.71 ± 63 and 36.19 ± 0.47 numbers. YAŞAR (2005) reported the Consumptionby all larval instars of *O. conglobate* increased on *Hyalopterus pruni* (Geoffroy) (Homoptera: Aphididae) The daily foodconsumption by adult females of *O. conglobate* in all petri dishes increased with increasingprey densities. Search rate according to life stage was significantly different but nodifferences occurred among 2nd, 3 and 4th instar larvae. Search rate decreased as life stage developed but increased when petri dish diameters increased .Ghadam (2009) *Oenopia conglobata* is one of the most active predators on forest trees and crop plants reported from Iran and the world. The eggs and the larvae were daily visited for the determination of hatching time and duration of different stages of development. The required period for development from eggadult in the temperatures of 15, 20, 25 and 30 OC were recorded 46.16, 26.46,16.86 and 13.78 days,respectively. The minimum thermal developmental threshold ranged from 6.44 and 12.83 0C for prepupa and 3rd larval instar, respectively. The threshold was 10°C for a complete life cycle. The degree- days of the latter were 270.32 days.

Alsamara and Okdah (2011) studied the effect of temperature on the growth of immature stages of the predator *Oenopia conglobata* L. (Coleoptera: Coccinellidae) and on the predation rate of larval instars. The prey was the nymphs of green peach aphid Myzus persicae (Sulzer) Results showed that the developmental time of the predator O. conglobata was affected by temperature and was on the two temperatures (25±2°c, 30±2°c) : (16.45±1.36, 22.5±1.8) days, respectively. Consumption rate of larval stages of O. conglobata was (18.60±2.44, 54.84±14.57, 101.50±16.09, 111.16±16.56) aphid nymphs for first, second, third, and fourth larval instars, respectively, on the temperature 25±2°c, and (23.06±3.08, 54.42±12.65, 102.57±12.61, 155.50±21.85) aphid nymphs on the temperature 30±2°c.

2.6. Situation of *Aleurocanthus spiniferus* in Apulia –Italy

The recent introduction of orange spiny whitefly (OSW), *A. spiniferus*in in Italy is a new challenge for whoever is involved in citrus producing chain activity (Kenawy *et al.,* 2014) . The first citrus infestation by *A. spiniferus* was recorded in the Lecce District (Fig. 9) in April 2008 (Porcelli, 2008) in a citrus backyard orchard. The pest was there at a low-level population for one to three years, without spreading. Later, by the end of 2008, *A. spiniferus* spread around, thus eliciting an alarm of the local growers. Countrywide collection activities showed that 13 municipalities in the Lecce district were invaded by *A. spiniferus* (Nutricato *et al.,* 2009): Alezio, Casarano, Collepasso, Gallipoli, Matino, Melissano, Parabita, Racale,Ruffano, Sannicola, Scorrano, Supersano and Taviano. At that time, *A. spiniferus* appeared as a major pest to many wild and economic plants; however, it was restricted to a small area of Puglia Region. At the end of 2009, the pest spread further infesting 68 of the 97 municipalities of the Lecce district with a various degree of infestation intensity. One year later, *A. spiniferus* infested 88 municipalities, but was still absent in Diso, Guagnano, Melendugno, Novoli, Salice Salentino, Squinzano, Trepuzzi, Uggiano, la Chiesa and Veglie. These nine uninfested municipalities are located along Brindisi-Taranto districts border, on the Adriatic coast, at the north edge of the infested area. During 2011, the pest spread into the villages alongside the Adriatic coast, only Diso and Melendugno being pest-free, apparently. Inspections in April 2011 showed that the pest outbreaks in San Pancrazio Salentino, a village in Brindisi district where *A. spiniferus* was recorded for the first time on citrus lemon in a private garden. In general, the older the infestation the higher the population; thus, the infestation level increased over time. Consequently, the most dense pest populations were found in the early infestation sites and at sea level. So, uninfested areas are green, low and moderately infested areas are yellow and orange, severely infested are red. OSW is a very polyphagous insect species and has been found in Rutaceae, Vitaceae, Araliaceae, Ebenaceae, Leguminosae-Caesalpiniaceae, Malvaceae, Lauraceae, Moraceae, Punicaceae and Rosaceae in Italy (Kenawy *et al.,* 2014).

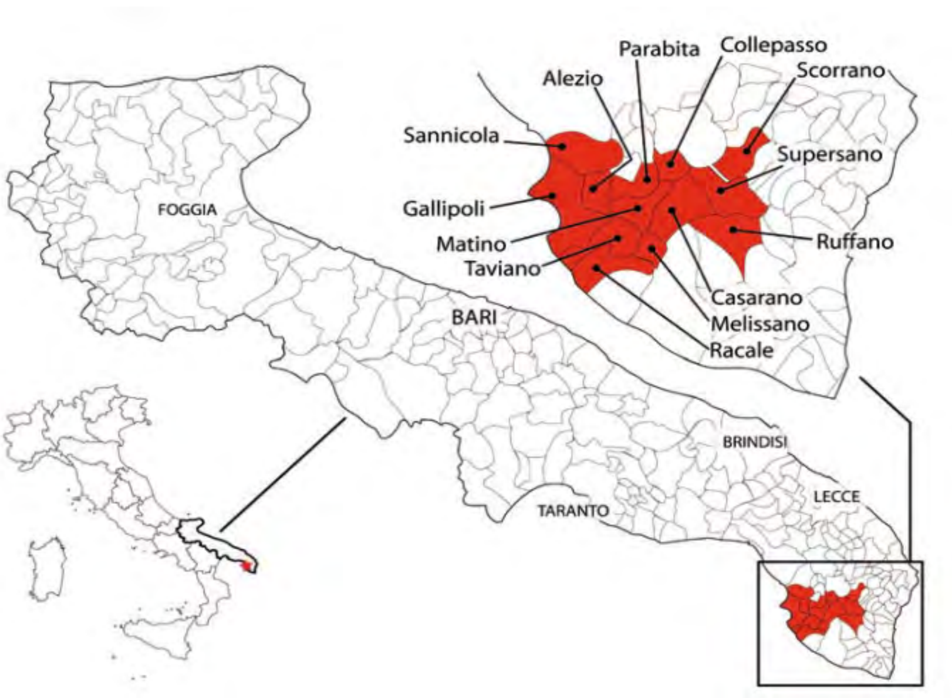


Image Credit: coffi et al,2013

Figure 9 First locations of citrus infestation by *A. spiniferus* at Lecce District

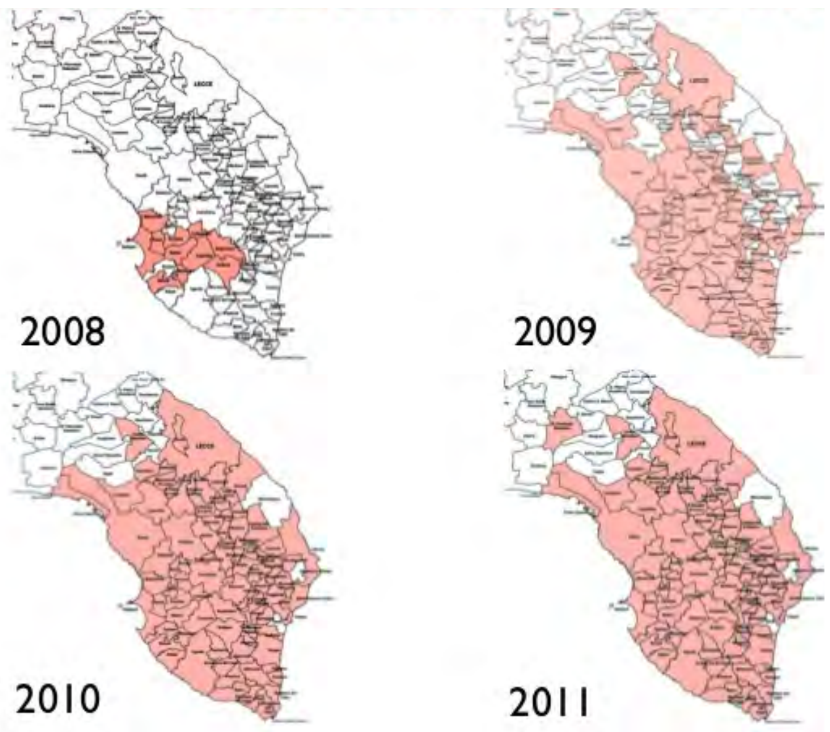


Image Credit: coffi et al,2013

Figure 10 Diffusion of *A. spiniferus* in Puglia in the period 2008-2011

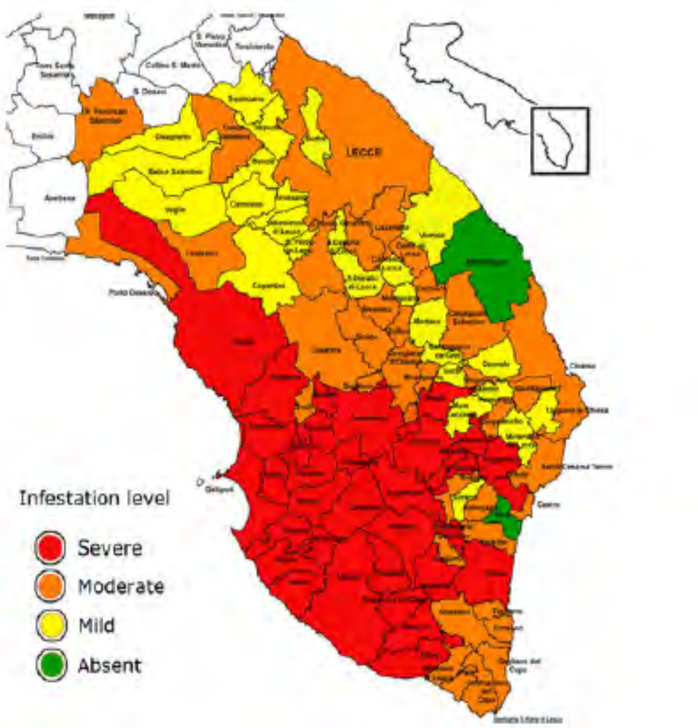


Image Credit: coffi et al,2013

Figure 2: Infestation levels of *A. spiniferus* in Puglia in the period 2008-2011

CHAPTER 3

3. Material and methods

3.1. Monitoring of *Aleurocanthus spiniferus* distribution in Apulia, Italy:

Infestation distribution of the invasive pest species, *A. spiniferus* was monitored in mostly 97 villages in the Apulia region, Italy for three years; 2013, 2014 and 2015. Monitoring and collection activity started 1st of March, 2013 and extended to 30th June 2015. A list of the sites explored was indicated on the map (Fig. 12). At each site, preferred host plants were inspected. Top leaves of tall plants were scrutinized backlight because black adults can be perceived from a relatively long distance. Sooty-mould blackened plants were also a good searching target for *A. spiniferus*, considering that the sooty-mould is due to honeydew excreted by diverse hemipteran species.

Figure 12: Sampling sites of *A. spiniferus* in Apulia

The plants in gas stations, urban private gardens and public parks, buses and railway stations were also examined. Urban and private gardens in old/ historical estate and villas were also considered to inspect for the presence of the pest (Cioffi *et al.,* 2013). Positive sites were visited several times during the three years of this study in order to confirm acclimation and the establishment of the pest. Few, but relevant confirmed data were given by citizens concerned about the bad appearance of private ornamentals or urban plants. During this monitoring, particular care was given to the move of OSW from invading areas to new ones. The species identification of the pest was confirmed through a slide mounting . A minimum number of infested leaves were collected and immediately sealed inside 5 cm large transparent cello-tape strips. This procedure allowed to move the collections from one site to another, with no dispersion risk. The yellow sticky traps were also used to check the presence of adults in the early stages of infestation.

Figure 13: Yellow sticky traps used for early detection of OSW infestation.

Monitoring was also carried out in September 2013 in Bari and Taranto districts. Beginning from the city center, monitoring was extended to the city boundary and countryside along the main routes, looking for infested host plant species. The samples were collected from various parts of the plants, starting from the middle area of the crown (shaded part) until the apical position the twigs. The pupal case, which are located on the underside of the leaf and mostly present on the ripe ones, was collected with the leaves and sealed between two pieces of clear tape (Scotch ®) 5 cm wide.

For the preparation of slides, some specimens were immediately immersed in 75% EtOH and stored in vials in liquid-tight. Adults and / or eggs (especially on young leaves) were collected in plastic bags (Ziploc®), and carefully sealed to prevent leakage and preserved. In the laboratory, pupae and pupae on the leaves were slide-mounted in Canada balsam as permanent microscopic slides according to the modified technique of (Watson & Chandler, 1999) and then labelled. For identification a stereomicroscope ZEISS, light compound microscope, equipped for bright field and phase contrast, and Hitachi TM3000 low pressure Scanning Electron Microscope (SEM), a stereomicroscope Nikon SMZ 800 and a compound microscope Olympus BX 50 were employed.

3.2. Survey of natural enemies associated with *Aleurocanthus spiniferus* in Apulia region

Sampling techniques employed in the agricultural fields were; visual counts (both destructive sampling and in situ counts of associated natural enemies on the crop plants), use of hand-lenses, entomological umbrella and sweep-net for arthropods. In the commercial fields, visual counts were conducted using a systematic sampling design, whereby a total of 15-25 plants per field per sample date were examined. These plants were sampled 3-5 transects within the field every 20 m for up to 80 m at each sample date. In addition, a 38-cm diameter sweep net was used for sampling arthropods on the crop foliage by using a prearranged M-shaped pattern, with 25 sweep-strokes per sampling point in the field. The sampling was carried out on trees of *Citrus* spp. and ornamentals plants placed on borders, in the middle of the field and in the backyards.

Photos and notes were taken on site, but the material sampled was brought to the laboratory in plastic bags and in plastic tubes taking care to seal the infested material in order to prevent the escape of adults during transportation. A camel hair brush, plastic cages, plastic bags, aspirators and alcohol 75% for conservation were used.

Following the same methods mentioned by Coffi *et al.* (2013), 10 sites located throughout the province of Lecce (Fig. 14) in both inland and along the Adriatic Sea and the Ionian Sea were sampled, here, where the selected locations; Copertino, Maglie, Botrugno, Ugento, Martano, Alezio, Gallipoli, Nardo, Supersano, and Salve. The sites were chosen at different ecosystem conditions to maximize the chances of finding natural enemies. All sites were monitored every 2 weeks during the study period for sampling collection.

The ornamental plants were the secondary hosts examined, while the *Citrus* spp. were the main hosts. At each plant, the underside of the leaves of the external part of the foliage and, in some cases, the internal part was the highly infested ones. The duration of the visual observations was the full day, and, by this way, the behaviour and the presence of the pest and its associated natural enemies at different temperatures during the whole day was represented. Sampling started the 1st week of May 2013 in the farmhouse “l’Aranceto” in Alezio in Lecce district. The field was located near a provincial road with a side of margin conterminous with the road.

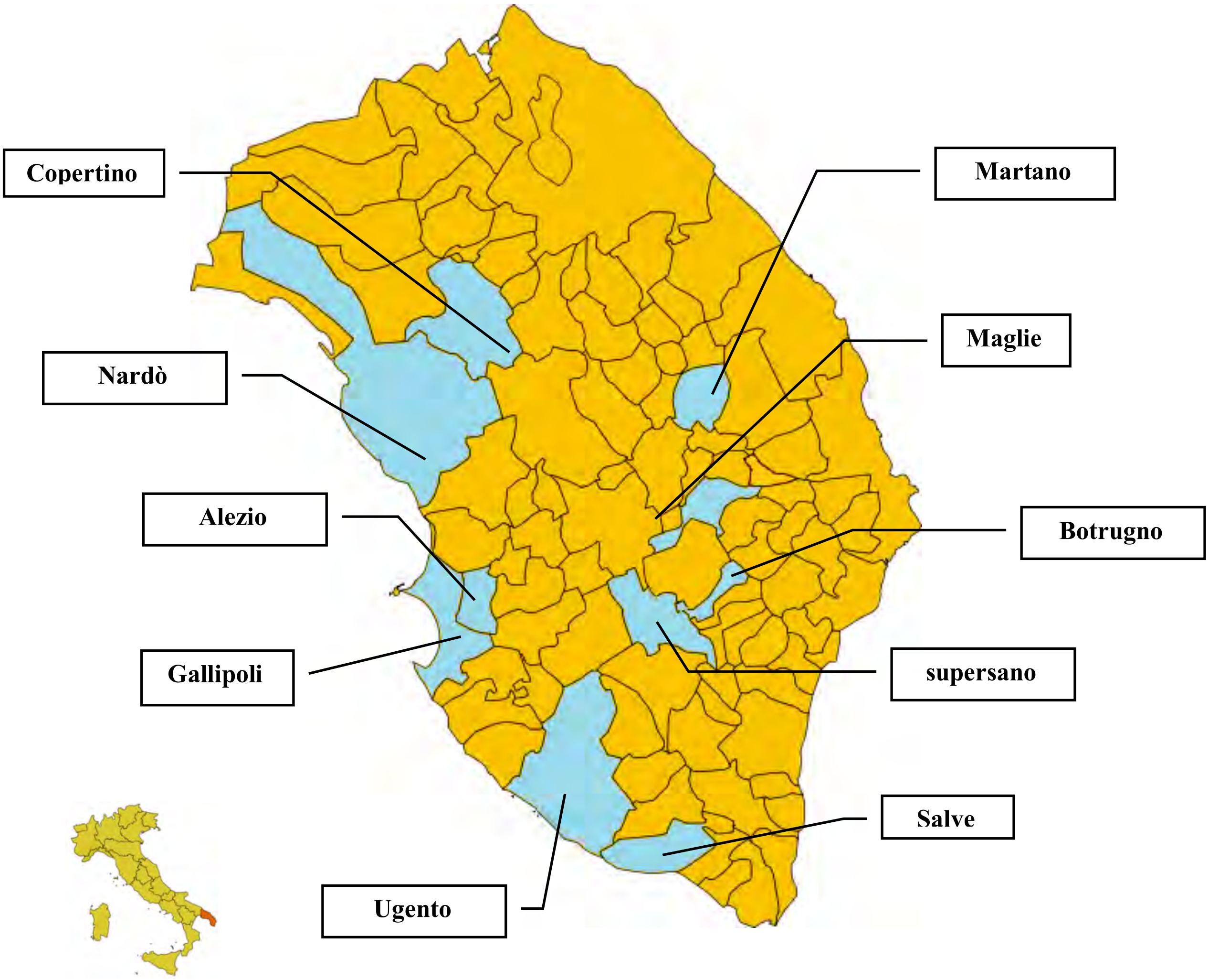


Figure 14: Sampling sites in the district of Salento.

3.3. Rearing of the natural enemies associated with *Aleurocanthus spiniferus* in Apulia

Collection, reference materials, key identifications and voucher specimens were used for specimen identification of the most important species found associated with *A. spiniferus* in Apulia. The laboratory works were carried out in a space of DiSSPA Dipartimento di Scienze del Suolo, della PIanta e degli Alimenti (about thirty square meters) with a single access, divided into two rooms, one for preparation and one for rearing, communicated through a door. The laboratory was used for the analytical and ancillary farming: receipt of specimens from the field, health assessment, preparation of the diet, *etc*, as well for the rearing of target species. The laboratory was well equipped.

3.3.1. Artificial diets

Trials to find out artificial diets for rearing the predators; *C. arcuatus, O. conglobate,* following the ones described by some authors, as patterns for rearing different ladybirds (Racioppi *et al.,* 1981; Hussein and Hagen,1991; Sighinolfi *et al.*, 2008, Muhammad and Saqib, 2010, Maryamand, 2011 and Morales-Ramos *et al.,* 2013), beside rearing the predators on the natural food, frozen *Ephestia* eggs were carried out.

3.3.2. Artificial diets prepared for rearing *Clitostethus arcuatus* and *Oenopia conglobata* :

- **Diet 1:** Test artificial diet (50% value honey+50% value yeast mixed with a small amount of pollen and diluted with distilled water). (Maryam Y, Mehdi Z, 2011)

- **Diet 2:** 25 g yolk, 2g sucrose, 49 g honey, 2 g casein, 1 g protein hydrolyzate were mixed thoroughly to form a uniform mixture (Muhammad and Syad, 2010).

- **Diet 3:** 1.3g agar, 16 g cane sugar, 6g honey and 1 g protein hydrolyzate dissolved in 100 ml of hot water and cooled to 35-38°C. 4.5g of royal jelly and stirred constantly until a homogenous white emulsion. The two mixtures combined with 0.5 g of alfalfa flour and yeast and 2g pulverized dry insect protein stirred vigorously and cooled to 5°C to begin solid (Muhammad and Syad, 2010).

3.3.3. Artificial diets prepared for rearing *Harmonia axyridis*:

- **Diet 4:** The compositions of the artificial diet (Hussein and Hagen, 1991 and Mirkhalilzadeh *et al.,* 2013.) are: 40 gr Chicken liver, 20 gr Baker's yeast, 20 gr Sucrose, 2 gr Agar, 100 mL Distilled water, 10 gr Egg yolk, 10 gr Honey, 5 mL Olive oil

- **<u>Diet 5:</u>** (Racioppi *et al.*, 1981 and Mirkhalilzadeh *et al.,* 2013).

  2.5 gr Agar, 150 mL Distilled water (mL), 12 gr Sucrose, 20 gr Honey, 5 gr Yeast extract, 3 gr Corn flour, 3 gr Soy powdered, 4 Milk (mL), 2.5 gr Gelatin, 0.5 gr Ascorbic acid, 5 gr Powdered milk, 1 gr Tryptophan, 1.5 Wheat germ oil (mL), 5 gr Vitamin Vanderzant, 0.06 gr Sorbic acid, 10 gr Sheep liver, 15 gr Egg yolk,Egg

- **<u>Diet 6:</u>** (Sighinolfi *et al.,* 2008, and Mirkhalilzadeh *et al.,* 2013).

  2.5 gr Agar, 150 mL Distilled water, 25 gr Chicken liver, 2.5 mL Corn oil, 3 mL Olive oil, 1.5 mL Glycerin, 0.3 gr Tryptophan, 0.5 gr Histidine, 1.5 gr Arginine, 5 gr Yeast extract, 1 gr Vitamin vanderzant, 0.06 gr Sorbic acid, 0.7 gr Ascorbic acid, 20 gr Sucrose, 15 gr Egg, 10 gr Powdered milk

Pollen (2 commercial bee pollen) added to the beetles separately without mixed with the diets.

Figure 153: Diet preparations.

Figure 16: Different artificial diets prepared.

3.4. Stock cultures of testing predator species

Adults of *C. arcuatus*, *O. conglobate*, were collected from a citrus farm in Lecce, Puglia, Italy. *H. axyridis* from a citrus field in Bari. The stock colonies of the predators were reared on the whitefly*, T. vaporariorum* and maintained in a growth chamber at 25±1°C, 70±10% RH, and a photoperiod of 16:8 (L:D) for at least two generations before being used for artificial diet studies.

- *C. arcuatus* and *O. conglobate* were reared on *T. vaporariorum*, maintained on tobacco plants at 25°C, 60-70% RH, and 16L:8D.
- *H. axyridis* adults were reared on *Ephestia kuehniella* eggs at the Faculty of Science, University of South Bohemia, Czech Republic for two generations before being reared on artificial diet.
- *H. axyridis* was reared on *Acyrthosiphon pisum*, maintained on faba bean plants at 25°C, 60-70% RH, and 16L:8D.

3.4.1. Rearing of *C. arcuatus* and *O. conglobate* on artificial diets:

- **Test artificial diet (1)** : Following the techniques described by Maryam Y, Mehdi Z, 2011, The artificial diet was applied dropwise onto a piece of board (0.5×5 cm) and placed in dishes during the tests. Newly emerged adult *C. arcuatus and O. conglobate* were kept in the containers described earlier, but were the fed different diets separately. Thus, a large culture of *C. arcuatus and O. conglobate* in containers was maintained on each of the diets separately.

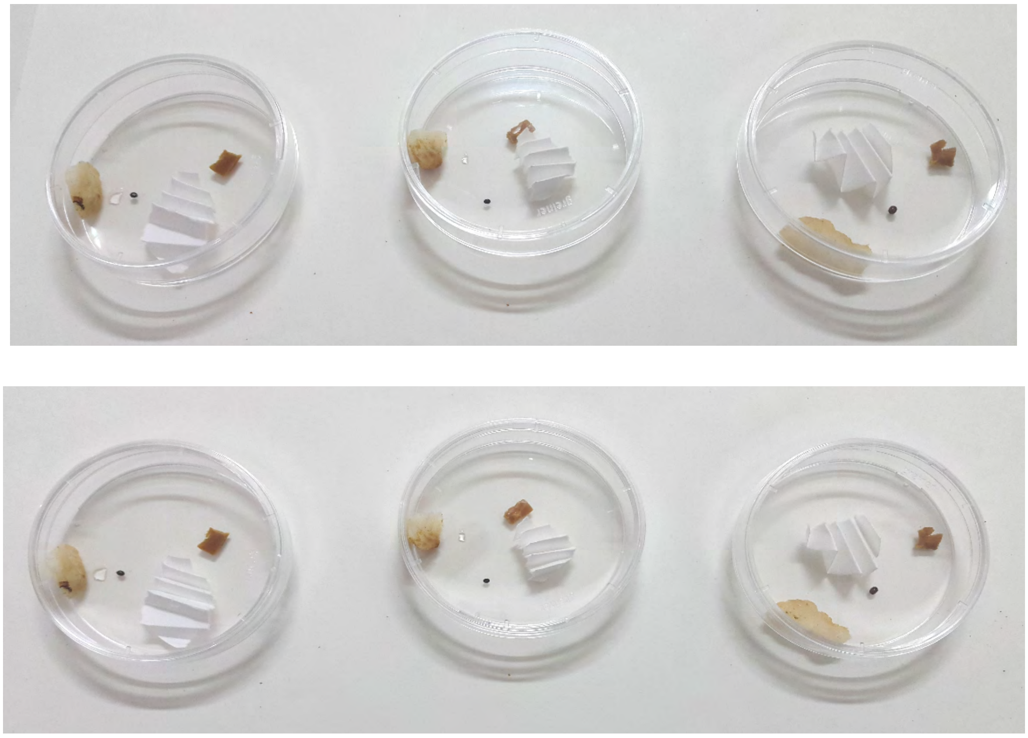


Figure 17: Rearing of *C. arcuatus* and *O. conglobate* on artificial diet 1

- **Test artificial diet (2)- (3):** Following the techniques described by Muhammad and Syad, (2010, all experiments were carried out on 1st larval instar. The nutrient components were poured into (6-cm) Petri dishes and then stored in freezer at -10 °C.

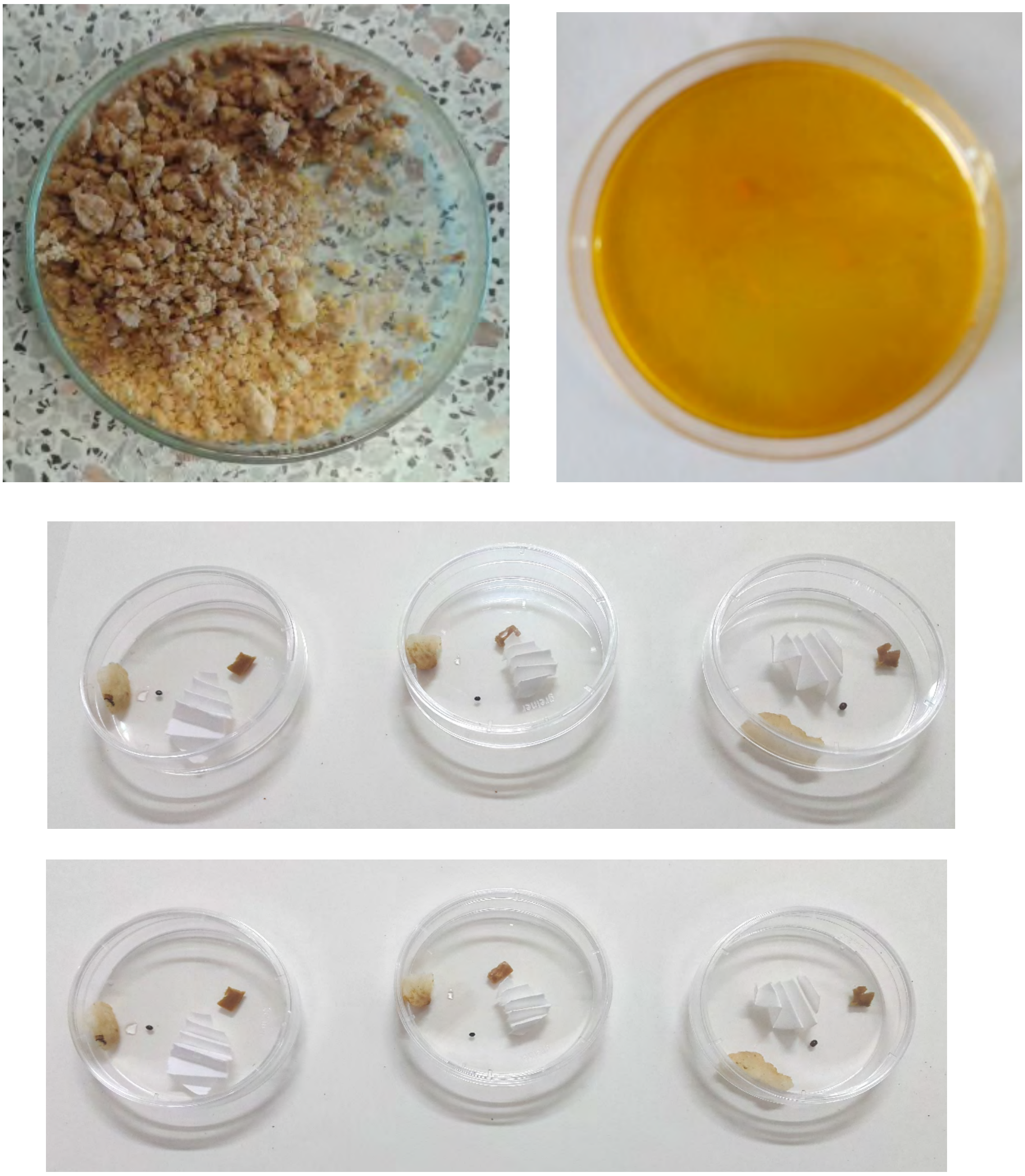


Figure 18: Rearing of *C. arcuatus* and *O. conglobate* on artificial diet 2-3

3.4.2. Rearing of *Harmonia axyridis* on artificial diet

Following the techniques described by (Racioppi et al. 1981; Hussein and Hagen,1991; Sighinolfi et al. 2008 and published by R.E. Mirkhalilzadeh et al 2013.Muhammad S and Syad M saqib, 2010, Maryam Y, Mehdi Z, 2011 and Juan A. Morales-Ramos et al. 2013)., all experiments were carried out on 1st larval instar. The nutrient components were poured into (6-cm) Petri dishes and then stored in freezer at -10 °C. Diet 6 showed the best primilary results

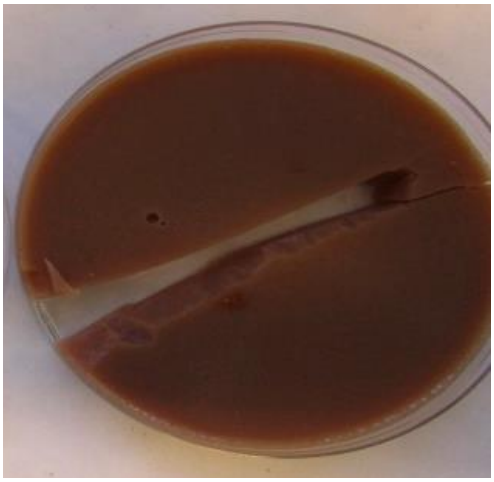


Figure 19: Diet 6 prepared for *H. axyridis* rearing

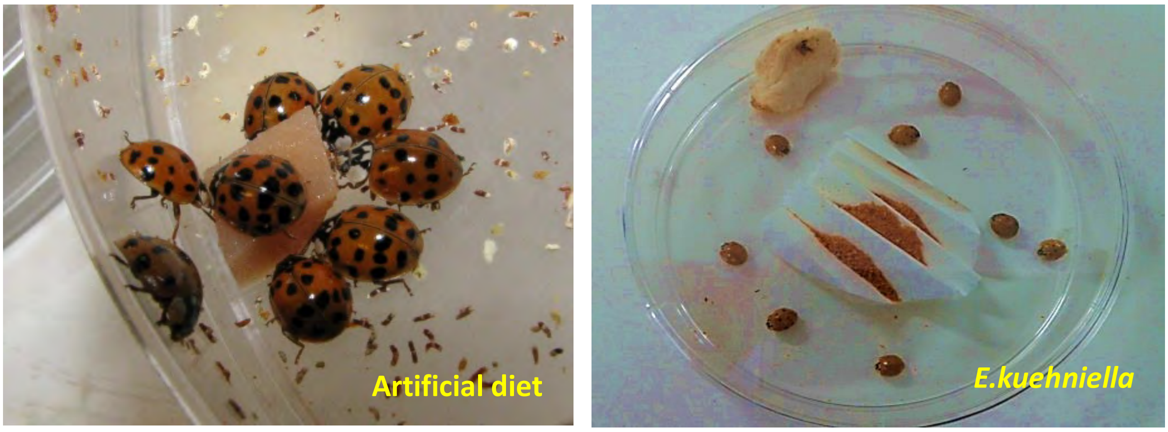


Figure 20: *H. axyridis* rearing on artificial diet and *Ephestia* eggs

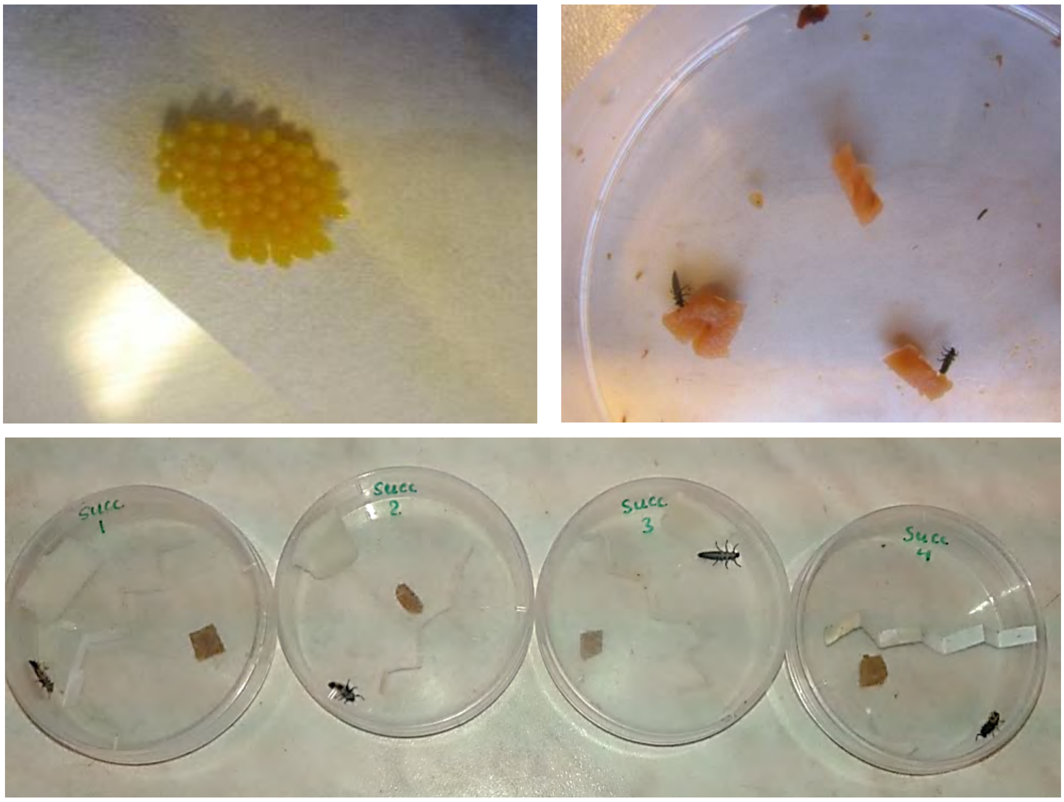


Figure 21: a) Egg cluster, b) Individual 2nd larval rearing on artificial diet and

Figure 22 : larve of *H. axyridis* feeding on artificial diet

❖ **Biological studies**

Following the techniques described by (Muhammad and Syad, 2010, Maryam,, 2011 and Mirkhalilzadeh *et al.,* 2013), all experiments were carried out on the 1st larval instar of each predator. The nutrient components were blended for diets 4-5-6 with agar at 45 °C, poured into 6-cm Petri dishes and then stored in a freezer at -10 °C. All experiments were carried out at 25±2°C, 65±5% RH and a photoperiod of 16:8 (L:D).

A couple of (female + male) of adult predator was released separately into a Petri dish contained the natural diet, each dish was checked daily for the deposited eggs for 30 days Until the death of female.

50 replicates for each strain were used, New laid eggs were transferred to new Petri dishes using a camel hair brush. The number of eggs was counted and kept in a sterilized blotting paper in small Petri dishes. Larvae were fed on an artificial diet along with pollens. The artificial diets were changed daily in all experiments. The incubation period was determined daily. Larvae were confined individually in Petri dishes to avoid cannibalism. Newly adult females and males were transferred to Petri dishes and supplied with artificial diet. The number of eggs laid, egg viability and adult mortality were recorded daily until all adults' death. The sex ratio was determined (N = 100 adults) reared on each diet using morphological characteristics to recognize the sexes. The developmental periods of immature stages of each species were calculated daily through larval moulting to the successive instars until pupation. Mortality rates of eggs, larval instars and pupae were recorded daily, number of eggs produced from each adult (fecundity) were counted and used to assess age specific reproduction parameters.

3.4.3. Evaluation of *Macrolophus pygmaeus* potential for the control of *Aleurocanthus spiniferus* egg:

Adults and nymphs of the predator were obtained from Verdepieno, Bioplanet company. Mass rearing was carried out using greenhouses whitefly *Trialeurodes vaporariorum* on ornamental Tabacco (*Nicotiana alata*) as prey. Under the laboratory conditions of (25±1°C, 70±5% RH, L16:D8), the predator was maintained in cages (25x 25x 20cm) containing a potted with citrus leaves infested with *A. spiniferus* .

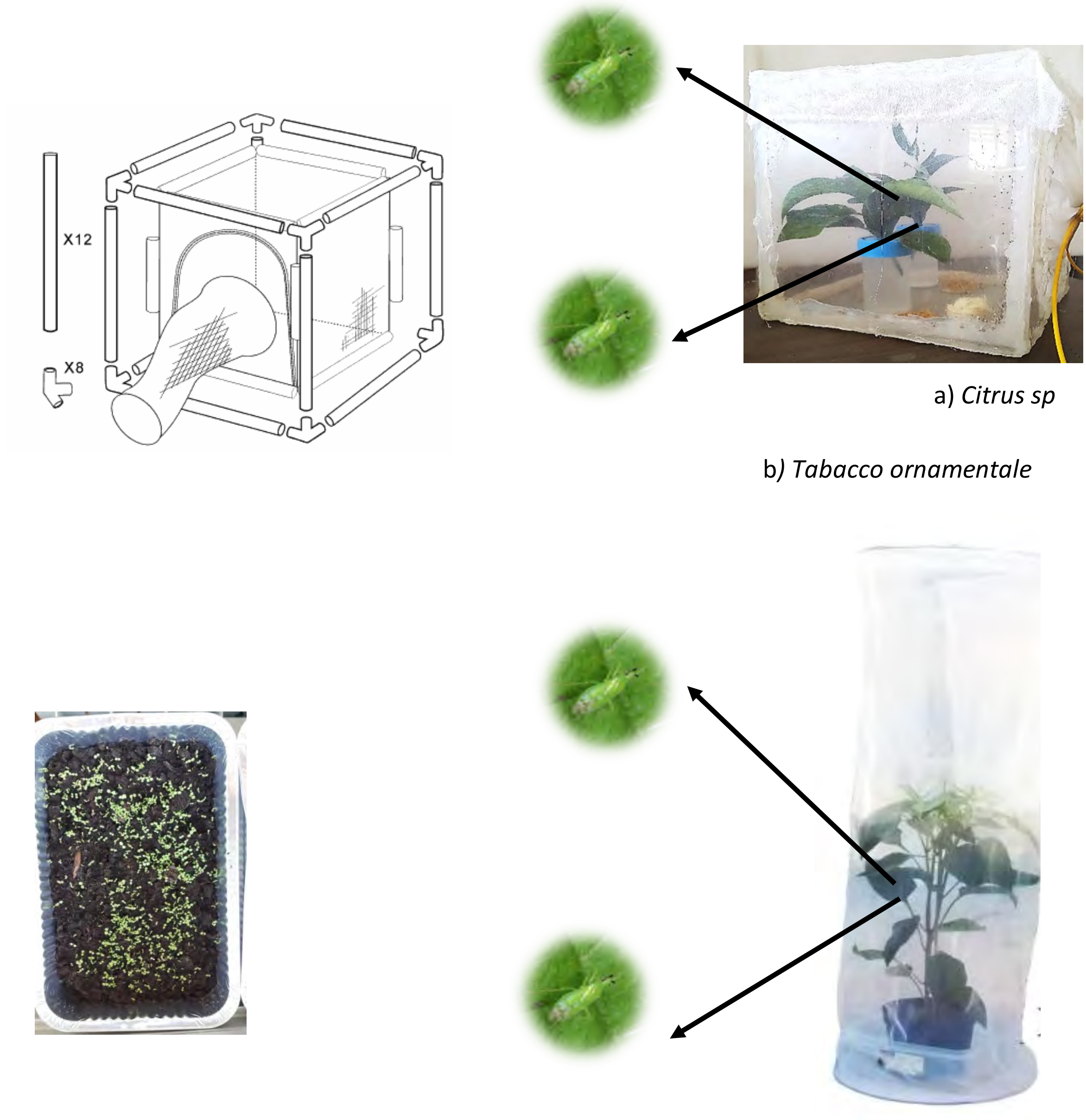


Figure 23: *M. pygmaeus* rearing on different host plants: a) *Citrus* sp. & b) Tabacco ornamentale (*Nicotiana alata*)

All plants were 15 cm high and had an average of 15 true leaves. Temperature and humidity were recorded by a data logger (Lascar Electronics, EL-USB 2).

Figure 24: Lascar Electronics, EL-USB 2, Eggs of *E. kuehniella*

3.4.4. Efficacy of *M. pygmaeus* on *Aleurocanthus spiniferus* eggs

All experiments were carried out in Petri dishes at 20 and 27ºC, 65±5% RH, and 16L:8D h photoperiod. Fifty replicates/ treatment were used and measurements were taken at 24-h intervals. To evaluate the efficacy of *M. pygmaeus,* 50 Petri dishes (60×15 mm), 25 for each experiment, contained 1 citrus leaf infested with fresh *A. spiniferus* eggs were used. A single *M. Pygmaeus* adult was placed in each Petri dish. The total number of eggs/ leaf was counted daily using a binocular.

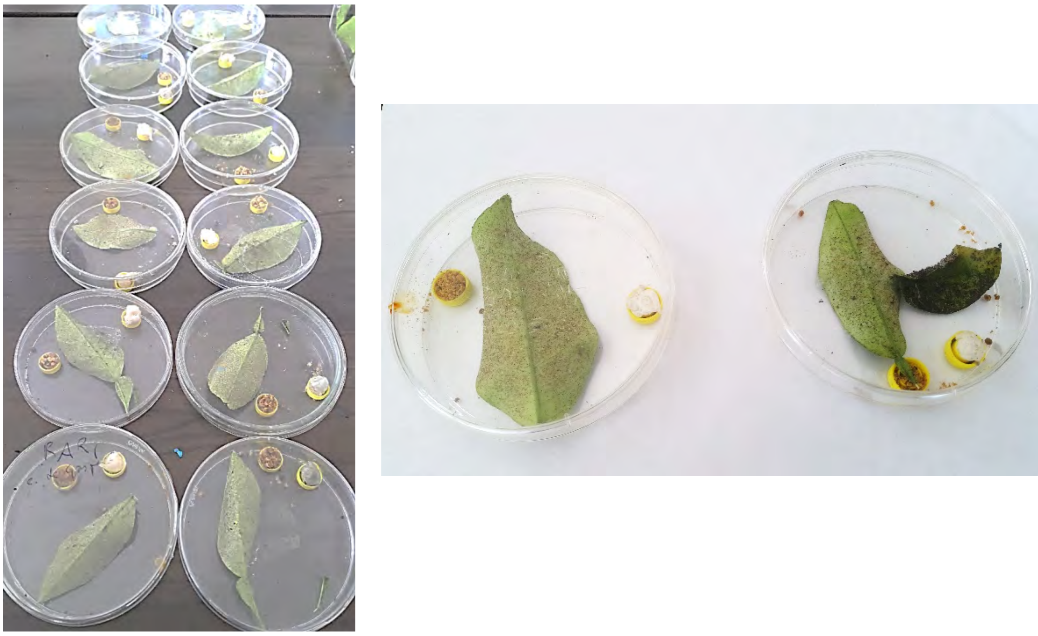


Figure 25 : A single *M. pygmaeus* adult was released per dish with OSW eggs on citrus leaves.

3.4.5. Efficacy of *Harmonia axyridis* against *Aleurocanthus spiniferus*

Citrus leaves infested with the immature stages of *A. spiniferus* were placed separately in Petri dishes (8 cm in diameter) with *H. axyridis* adults at the laboratory conditions of (25°C and 60% RH) for 24 hours, 30 replicates were used one adult/ Petri dishes . Newly infested citrus leaves were replaced every 48 hours. Daily inspections of the leaves in the Petri dishes were done using a stereomicroscope for two hours in the morning (10:00 to 12:00) and two hours in the afternoon (15:00 to 17:00) to estimate the efficacy of *H. axyridis* adults.

Fig 26 : Efficacy of *Harmonia axyridis* against *A. spiniferus* egg/nymph

3.5. Field release trial of *H. axyridis* in citrus grove

A field release experiment of *H. axyridis* was carried out in a citrus grove infested with *A. spiniferus* (400^2 meters), located at the Faculty of Agriculture, –University of Bari, Italy in 2015.

Figure 27: Felid release experiment of *H. axyridis* in a citrus grove infested with *A.spiniferus* in 2015.

The experimental field was divided into six plots, each (66 m^2). Three, were considered experimental plots and three were the control (predator-free). The citrus trees in the grove were cultivated at 2 m and 3 m space between and within the trees, respectively. The release took place from 3th May to 22nd July 2015. The release rate was 100 adult per plot, a total of 300 predators.

Laboratory laid and emerged eggs and nymphs of rearing predators, respectively

3.6. Statistical analysis

Data were statistically analyzed using the factorial ANOVA using SAS Analytics.

Chapter 4

4. Results and Discussion

4.1. Field Studies

4.1.1. Monitoring of *Aleurocanthus spiniferus* (Quaintance, 1903) spread in Apulia region:

A total of 97 sampling of *A. spiniferus* included 97 Sites and 88 host plants were inspected in Bari and Toronto city, during the period 2012-2015. Data were summarized in Annex (1). In 2012, neighboring municipalities were monitored. In San Pancrazio Salentino (one municipality showed positive results in 2011 on the border between the province of Lecce and those of Taranto and Brindisi) with negative results for the presence of *A. spiniferus*. The presence of the pest was recorded in 2014 in Brindisi and in the municipality of Torchiarolo on *Citrus* plants.

The monitoring was carried out in the city of Bari in 2014, showed two positive sites; in Amendola Street on *Parthenocissus tricuspidata* and *Citrus*, one in Padre Pio Street on *Parthenocissus tricuspidata*, one in a public garden in Viale della Repubblica on *Rosa,* and one in Nizza Street on *Vitis vinifera* and in Orabona Street in Universitary Campus on *Citrus*.
In 2014, it was also recorded in Corso Alcide de Gasperi in a *Citrus* garden, instead in the province of Bari in 2013, where all sites were negative. The only positive result was in 2014 was on the way to Noicattaro Torre a mare (Kenawy *et al.,* 2014).

The monitoring carried in 2014 in the city of Taranto was positive in Ciro Giovinazzi Street on *Citrus* spp, *Parthenocissus tricuspidata, Rose, Citrus* spp.. In 2014, the pest was found in 5 municipalities of the province of Taranto (Monteparano, Fragagnano, Manduria, Sava, Avetrana). In 2014 also, it was reviewed in Diso and Melendugno in the province of Lecce and the results were negative, but it was observed in a high presence on *Citrus*.

4.1.2. Survey of the common natural enemies associated with *Aleurocanthus spiniferus*

A survey of the most common and abundant natural enemies, mostly predators and parasitoids, associated with *A. spiniferus* was carried out in Apulia region, Italy from April to October 2013- 2015, revealed the presence of 5 species of predators in the area; 4 coccinillids and one chrysopid. Locality, period of occurrence and relative abundance are summarized in table (2).

Table 10: Survey of the common and abundant predators associated with *A. spiniferus* in Apulia region 2013-2015

Species	Period	Observed stage	Abundance	Location
***Clitostethus arcuatus* (Rossi) (Coleoptera: Coccinellidae)**	April to mid--October	Larvae	++	Lecce district Bari district
***Oenopia conglobata* (L.) (Coleoptera: Coccinellidae)**	Mid-f April to September	Adults Larvae	++	Lecce district Bari district
***Scymnus sp.* (Coleoptera: Coccinellidae)**	May to mid-October	Adults Larvae	++	Lecce distract Bari district
***Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae)**	May to October	Adults Larvae	+	Lecce district Bari district
***Chrysoperla carnea* (Neuroptera: Chrysopidae)**	June to October	Adults	+	Lecce district

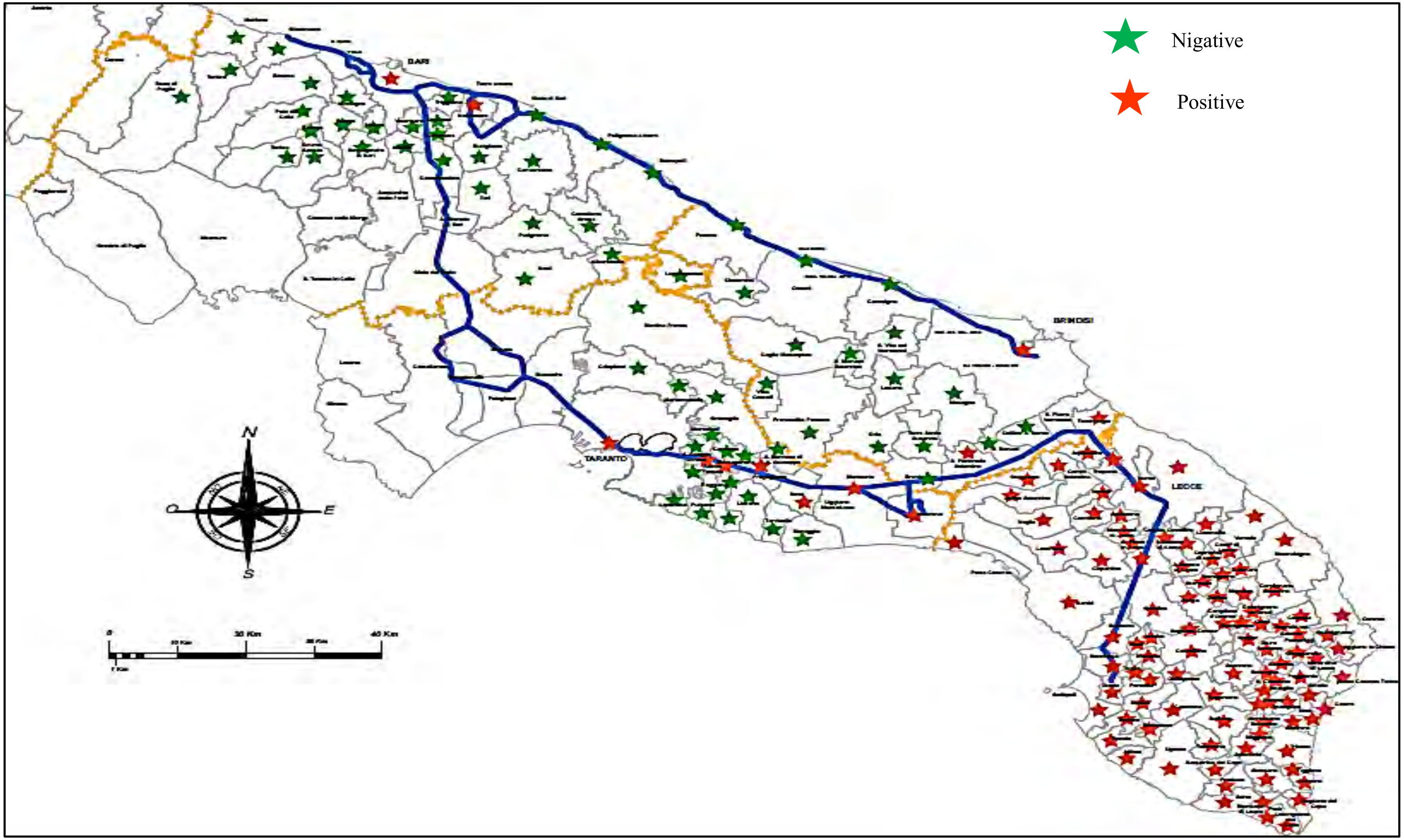


Figure 28 : Spread of *A.spiniferus* in Apulia region 203-2014-2015

4.1.3. Release and evaluation of the efficacy of predators in the field:

4.1.3.1. Release of *M. Pygmaeus* against *Aleurocanthus spiniferus*

Adults and nymphs of *M. Pygmaeus* were released two times; first one by mid-July and the second was in November to evaluate their efficiency against *A. spiniferus*. The percentage of damaged leaves by *A. spiniferus* was significantly higher. Insignificant differences were found in the percentages of damaged leaves with the presence of pests when *M. pygmaeus* was present in the field due to the heavy infestation and the presence of the sooty mould.

In some studies, the predator *M. Pygmaeus* reproduced well when attacking white flies, but it did not do under the given experimental conditions in the open field. *M. pygmaeus* performed poorly on *A. spiniferus* in open field may be due to the following reasons: 1) It is very sensitive to high temperatures as it was in July (more than 32ºC) although few nymphs and adults were found after 2 weeks from the release, and 2) The heavy infestation and the presence of the sooty mould blocked the searching capacity of the predator. So that, it's recommended to use *M. pygmaeus* under the controlled conditions such as greenhouses, but if it is necessary to release it in the open field, it's recommended to be relatively in mild temperatures. although the ability of *M. pygmaeus* to complete its development feeding on *A. spiniferus* was not checked, we showed that at least in the short term, *A. spiniferus* could be a suitable prey for M. pygmaeus. The predatory efficiency of adults/5th nymph was evaluated because they make use of new prey resources.Therefore, these results cannot be generalized without further study.

4.1.3.2. Release of *Harmonia axyridis* against *A. spiniferus:*

In Apulia 2015, one adult individual of *H. axyridis* was found and photographed on a branch of an ornamental tree (Bignoniaceae) in a green area of Monopoli (Bari province) MENCHETTI *et al.,* (2015). *H. axyridis* is a polyphagus predator of aphids, but as it was found on the citrus leaves infested only with OSW, that may be an indication of accepting it as a prey for *A. spiniferus*. Further field studies on the predator preference and efficacy against OSW are still needed.

Figure 29: *Harmonia axyridis* adults released and feed in on citrus leaves infested with *A.spiniferus*

4.2. Laboratory studies

4.2.1. Rearing of *Harmonia axyridis* on artificial diet on Diet 6 (Sighinolfi et al., 2008, and Mirkhalilzadeh et al., 2013).

4.2.1.1. Effect of strain and artificial diet on body weight

As shown in the table, there was a significant difference (,*f* =57.3, *p*<0.000 and *f* =103.6, *p* <0.000*) between the 2 strains used (Table 3) . There was also a little significant difference between both sexes, as the females were bigger (*f* =5.0, *p*<0.028*). There were interactions between strain and diet (f =6.9, p <0.011*) and sex and diet (*f* =2,1,*p* <0.154) and strain*sex (*f* =7,1,*p* <0.010*).

Table 11: Effect of the artificial diet on body weight of*Harmonia axyridis* (2 strains)

	Adult weight (mg)			
	H. axyridis succinea		*H.axyridis* bifasciata	
	M	F	M	F
E.kuehniella	36.13	43.5	27.6	28.7
Artificial diet	23.27^{a}	27.11^{b}	20.6^{a}	18.6^{b}

4.2.1.2. Effect of strain and artificial diet on fecundity and fertility

There was a strong effect of diet females as they had less fecundity (Table 4) strain (f =1.06,p<0.311), diet (f=65.31,p<0.000*) and the interaction between strain and diet had a strong effect on strain succinea but little on strain bifasciata (f =8.17,p<0.008*). There was a little effect of diet and of interaction as in fecundity. It was strong in diet females that had less fertility strain (f= 0.16,p<0.692), diet (f =69,73,p<0,000*) and interaction between strain and diet (strong effect on succinea but small on bifasciata (f =15.92, p<0.000*) (Table 4).

Table 12: Effect of strain and diet on fecundity and fertility of females:

Food type/ Strain	***H. axyridis* succinea**		***H. axyridis* bifasciata**	
	Fecundity	Fertility (%)	Fecundity	Fertility (%)
E.kuehniella	24.7	25.1	24.3	19.7
Artificial diet	12.3	9.9	17.4	14.3

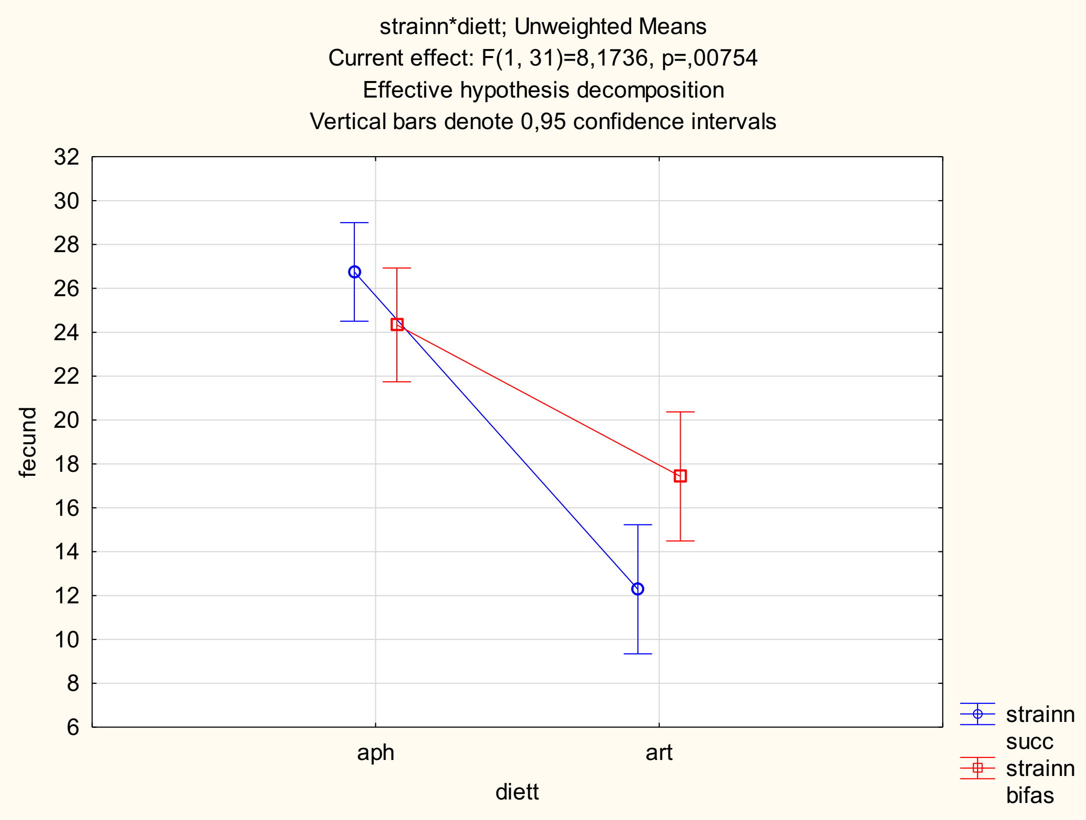


Figure 4 : Effect of strain and diet on fecundity of *H. axyridis* suc., bif. females

4.2.1.3. Effect of strain and artificial diet on pre-oviposition and incubation period

Both diets and strains had a strong effect on the pre-oviposition time (Table 5). It was much longer in case of the artificial diet; strains (f=60.8,p<0,000*) and diet (f=284.2,p<0,000*), and was longer in bifasciata strain than in succinea. There was no interaction between strain*diet (f =0.3,p<0,606), so that reaction of both strains was similar. Only diet affected the incubation period (longer) but the two strains did not differ.

Table 13: Effect of strain and diet on pre-oviposition and incubation period

	***H. axyridis* succinea**		***H. axyridis* bifasciata**	
	Preoviposition period/days	Incubation period/days	Preoviposition period/days	Incubation period/days
E. kuehniella	6.2	5	11.6	4.9
Artificial diet	17.4	8.8	22.1	9.4

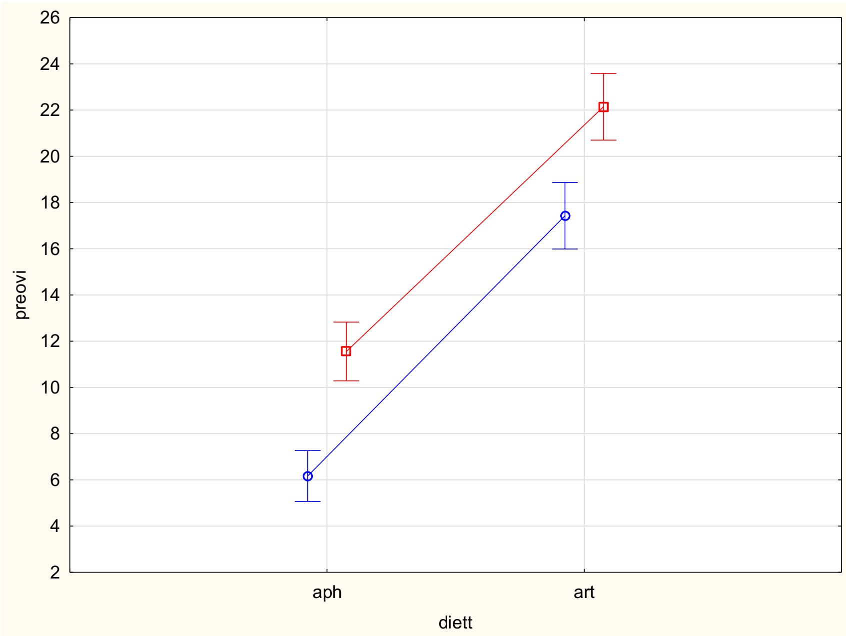


Figure 5 : Effect of strain and diet on pre-oviposition period of *H. axyridis* suc., bif. Females

4.2.1.4. Effect of strain and artificial diet on larval developmental time

Effect of strain and diet on developmental time (Table 6) of the 1st instar, both differed as the development of succinea strain and feeding on artificial diet lasted longer. Strain (f =377.3, p<0.000*), diet (f= 353.3,p<0.000*). There was an interaction, as the effect of diet was stronger in succinea strain*diet (f =9.8,p<0.002*). For the 2nd instar, development on artificial diet lasted longer strain (f =38.5,p<0.000*), diet (f =376.1,p<0.000*). There was an interacton strain*diet (f =72.8,p<0,000*), as the effect of diet was stronger in succinea, lasted longer only on poor artificial diet. In the 3rd instar, there was only an effect of diet (f =67.43,p<0.000*), as it was longer, while the strain did not differ (f =3,29,p<0.073), strain*diet (f =2.32, p<0.131). Effect on the 4th instar (excluding prepupa), both differed (f =30,63,p<0,000*) as the development of bifasciata strain and on artificial diet lasted longer (f =52,07,p<0,000*). There was an interacton, (f =8,01,p<0,006*) as the effect of diet was stronger in bifasciata.

Table 14: Effect of strain and diet on larval instar developmental time (in Days) of *H. axyridis* strains

	Larval development time							
	H. axyridis succinea				*H. axyridis* bifasciata			
	1st	2nd	3rd	4th	1st	2nd	3rd	4th
E.kuehniella	4	4	5.15	5.9	3.1	4.2	5.1	6.7
Artificial diet	5.17	6.89	7.36	7.26	4	5.4	6.6	9.9

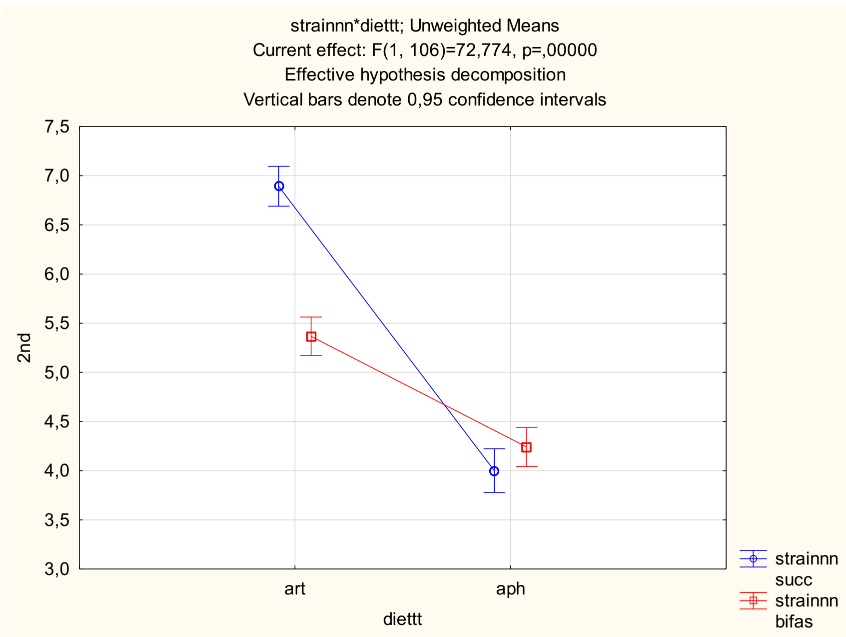


Figure 32: Effect of strain and diet on 2nd larval instar developmental time (in Days) of *H. axyridis*

4.2.1.5. Effect of strain and artificial diet on pupal period

There was no effect of either diet or strain (f =0.076,p<0.783), (f =0.076,p<0,783), only interaction (f=5.923,p<0.017*). In bifasciata strain, development lasted shorter, while in succinea strain, development time was longer, followed feeding on *E. kuehniella*.

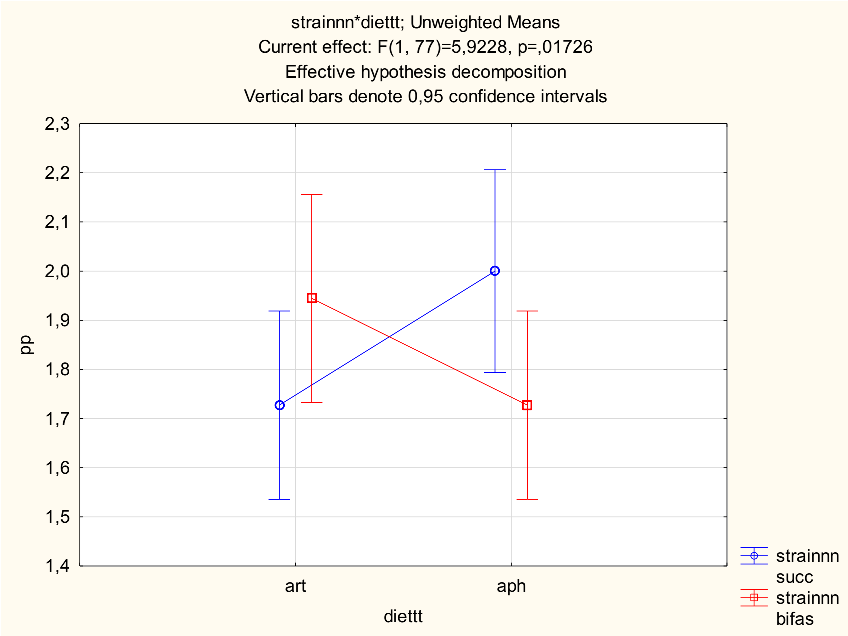


Figure 6: Effect of strain and artificial diet on pre-pupa period

For the pupae, both factors had strong effect and were in strong interaction (f 21,79,p<0,000*): bifasciata had much longer (f 33,55,p<0,000*) development time after feeding on artificial diet (f 16,35,p<0,000*), while succinea pupae were not affected.

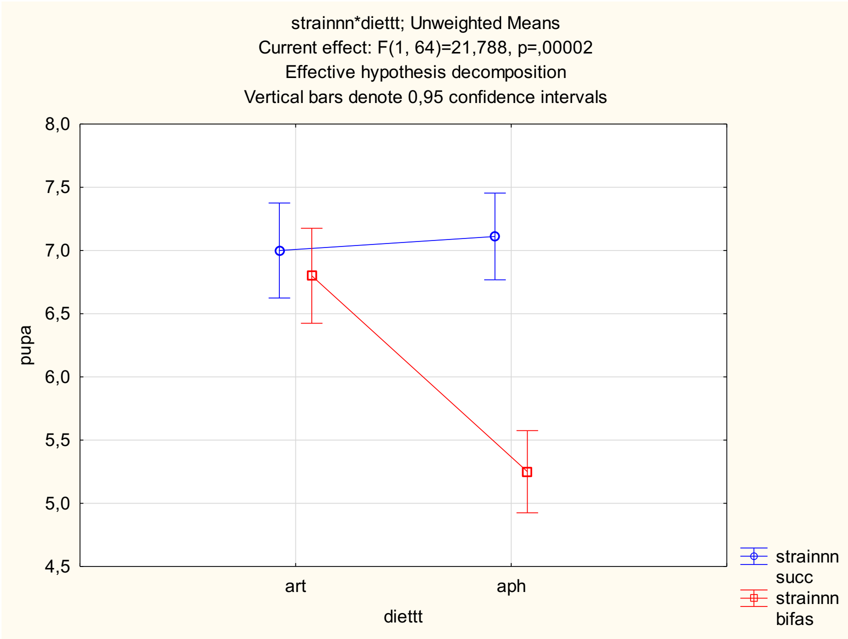


Figure 34: Effect of strain and artificial diet on pupa period

4.2.1.6. Effect of strain and artificial diet on total developmental period

For the entire post-embryonic preimaginal development, strain (f 5.7,p<0.020*) diet (f 297.6,p<0.000*) had a strong effect (longer on artificial diet), while strain and interaction strain*diet (f 5.2,p<0.026*) had less significant effects: longer development of science on *E. kuehniella*, and equal development time on artificial diet.

Table 15: Effect of strain and diet on total preimaginal developmental time

Developmental duration (Day)(Egg-Pupa)		
	H. axyridis succinea	*H. axyridis* bifasciata
E.kuehniella	28.2±0.4	26.2±0.6
Artificial diet	35.4 ± 0.1	34.5±1.4

4.2.1.7. Effect of strain and artificial diet on fecundity and sex ratio

Table 16: Fecundity (total no. of eggs) and sex ratio of *H.axyridis*

	***H. axyridis* succinea**			***H. axyridis* bifasciata**		
	Sex ratio (%)		Total eggs	Sex ratio (%)		Total eggs
	M	F		M	F	
E. kuehniella	10	13	321	9	10	219
Artificial diet	9	7	86	9	6	122

4.2.1.8. Effect of strain and artificial diet on mortality (%)

Only diet (f 175.6,p<0.000*) had an effect (longer on artificial)(f 2.3,p<0.144), but males and females did not differ. diet*sex (f 1.2,p<0.276). For bifasciata, the longer the development, the smaller the adult.

Table 17: Mortality % of *H. axyridis* succinea

	Mortality %					
	Larval instars					
	1st instar	2nd instar	3rd instar	4th instar	Pre pupa	Pupa
E.kuehniella	0	3.4	7.4	3.8	0	4.2
Artificial diet	0	7.1	12	8.7	4.5	6.7

Table 18: Mortality % of *H. axyridis* bifs

	Mortality %					
	Larval instars					
	1st instar	2nd instar	3rd instar	4th instar	Pre pupa	Pupa
E.kuehniella	0	3.4	11.5	13	4.5	10
Artificial diet	0	0	20	19	27.8	20

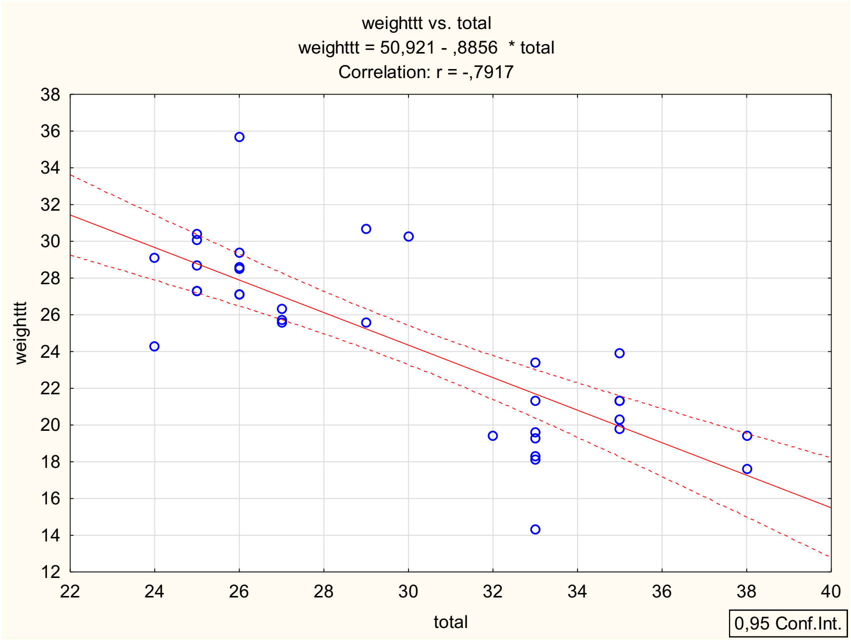


Figure 35: Effect of strain and artificial diet on the interaction between the weight and totale egg of *H. axyridis*

4.3. Morphological comparison of *H. axyridis* succinea reared on *E. kuehniella* or artificial diet

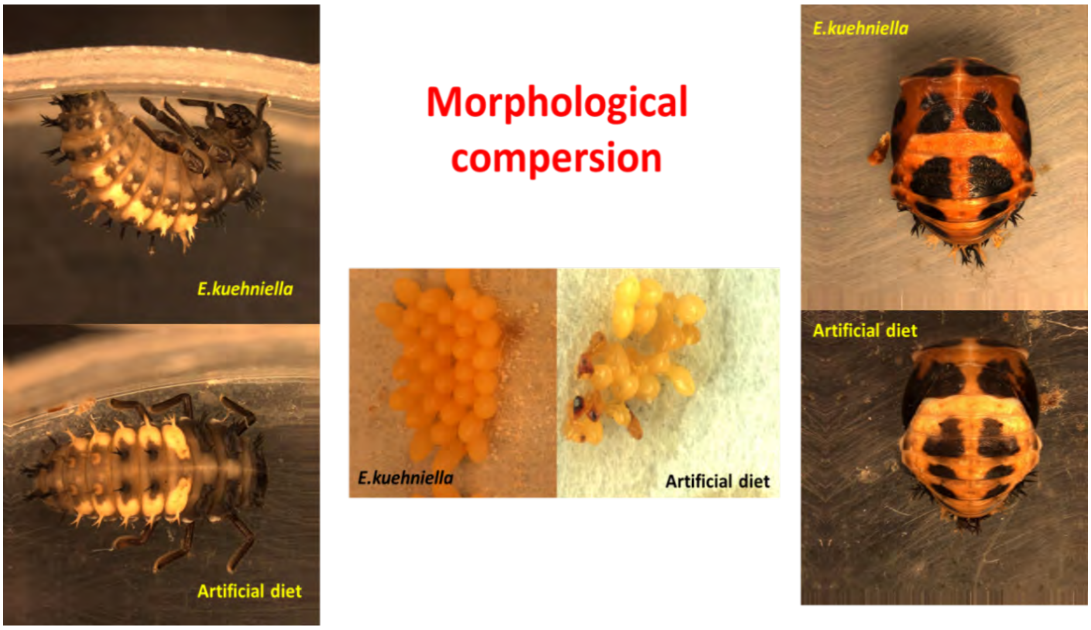


Figure36 : Morphological comparison of *H. axyridis* succinea reared on E. kuehniella or artifici al diet

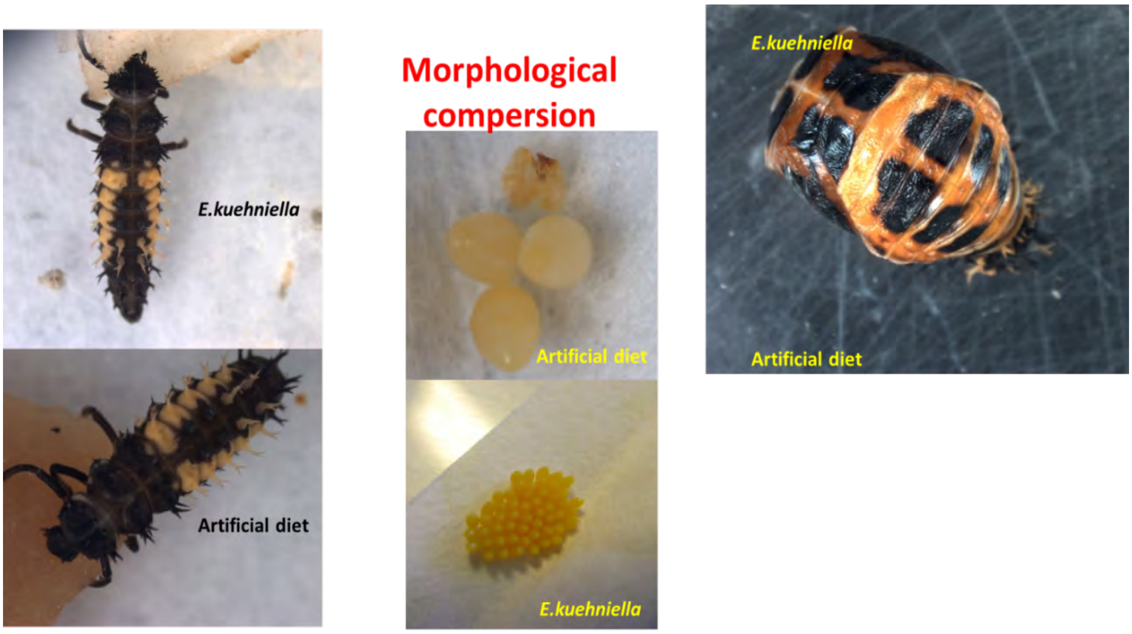


Figure 37: Morphological comparison of *H. axyridis* bifas. reared on *E. kuehniella* or artificial diet

4.4. Rearing of *C. arcuatus* and *O. conglobate* on artificial diet 1-2-3

The diets described by Yazdani and Zarabi (2011) (Diet 1) and Sarwar and Syed (2010) (Diet 2) were used for rearing *C. arcuatus* and *O. conglobate,* respectively.

Diet (1)
Yazdani and zarabi, 2011

Diet (2)
Sarwar and Syed, 2010

Diet (3)
Sarwar and Syed, 2010

Figure 38: prepared artificial diets for Rearing of *C. arcuatus* and *O. conglobate*

Four trials were carried out to rear the 2 predator adults on artificial diets, 3 failed to obtain eggs. As well was the case for the 1st larval instars.

Many factors in the rearing process could affect the performance of *C. arcuatus and O. conglobate* on artificial diet, e.g., proportions of different nutritional ingredients, physical conditions of the diet (including moisture levels and irregularities of the diet surface), microbial contamination, several specific compounds, and environmental conditions in culture rooms. The main protein sources. Fat soluble vitamins and other substances mised in diets might also have an effect on predators.

4.5. Efficacy of *Macrolophus pygmaeus* potential against *Aleurocanthus spiniferus* eggs

Fifty replicates were used daily to examine the consumption rate of the predator. The results showed that *M. pygmaeus* adults and nymphs were efficient in consuming of *A. spiniferus* eggs. The results presented in table (12), the consumption reached an average of 50-54 egg/day/predator adult respectively .

Table 11: Weekly average consumption of *M. pygmaeus* adult and last nymphal instar on *A. spiniferus* egg at two differant temperatures

Weeks/ stage	22 °C		27 °C	
	5th instar	Adult	5th instar	Adult
1st week	10.08±2.0	10.28±0.3	50.60±2.2	54.8±1.7
2nd week	16.44±1.4	23.01±0.5	49.21±0.87	50.12±0.9
3rd week	14.63±1.3	18.45±0.5	48.17±0.81	48.04±0.8
4th week	14.78±1.3	24.27±0.4	47.13±0.80	47.26±0.7
5th week	12.19±1.4	13.53±0.5	47.23±0.87	47.8±0.8
6th week	13.66±1.2	15.02±0.3	44.71±0.70	43.95±0.6

The temperature was found to have an effect on the rate of predation of both adults and nymphs of *M. pygmaeus* feeding on *A. spiniferus* eggs. The difference in predation rate among the adults and the nymphs was highly significant. There were significant interactions between temperature and predator stage. The temperature was found to have an effect on the rate of predation of both adults and nymphs (f 5342,p<0,0001) of *M. pygmaeus* feeding on *A. spiniferus* eggs. The difference in predation rate among the adults and the nymphs was highly significant (f 33,p<0,0001) There were significant interactions between temperature and predator stage (f 26,p <0,0001).

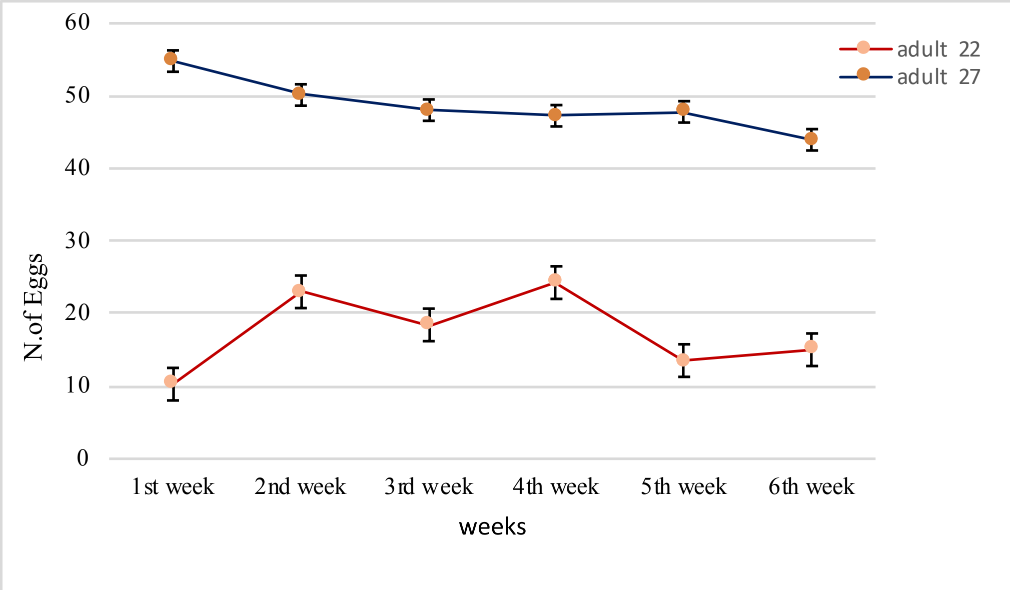


Figure 39: Weekly average consumption of *A. spiniferus* eggs by *M. pygmaeus* adult at two different temperatures (22° c -27° c)

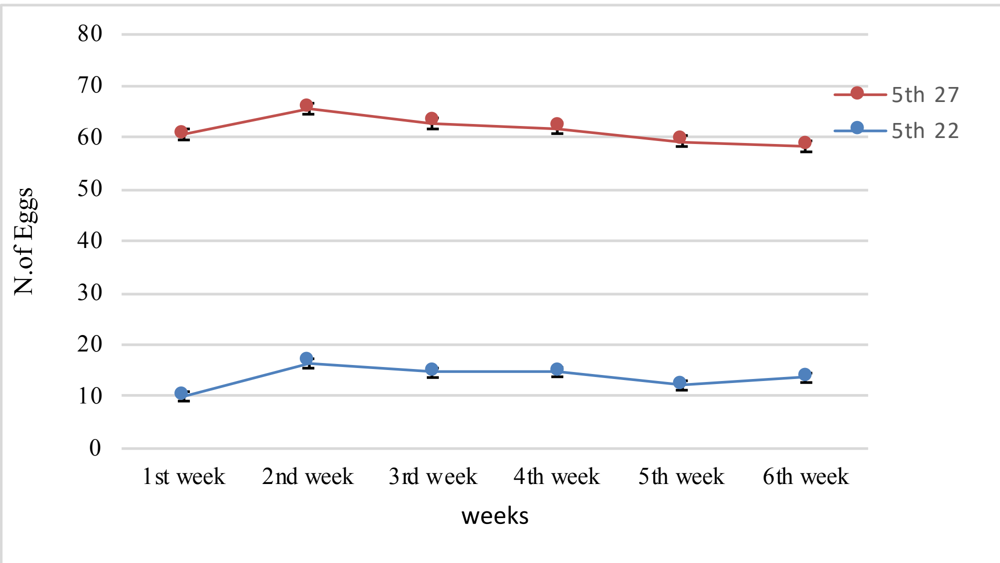
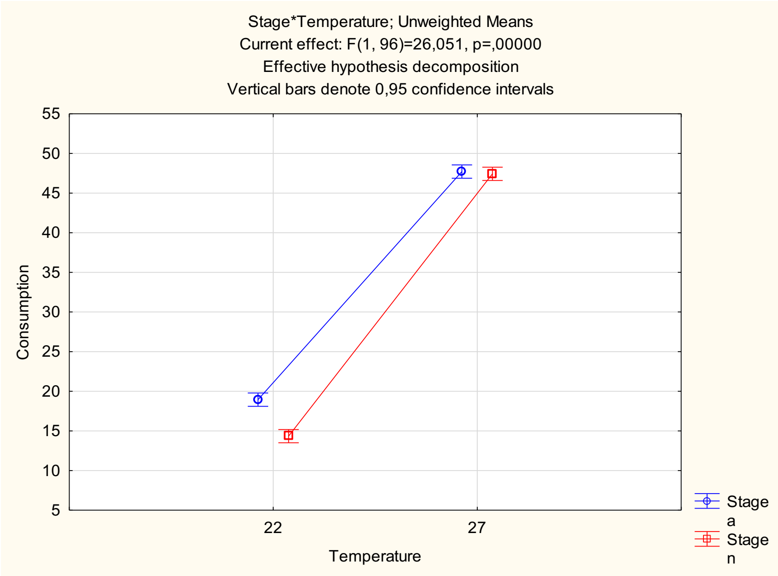


Figure 40: Weekly average consumption of *A. spiniferus* eggs by *M. pygmaeus* 5^{th} nymphal instare at two different temperatures (22° c -27° c)

In both developmental stages, consumption increased with the increase in temperature. It was three times more in nymphs than in adults.



Although the changes of consumption rate during the experiment was not perfectly linear, but it followed some fluctuations up and down

Figure 41: consumption of *A. spiniferus* eggs by *M. pygmaeus* adult

New foci in Bari, city center and province, Taranto have been detected, showing the high, spreading capability and the harmfulness of this pest for the whole agriculture, and the urgent need for a setting up of control strategies. It is very important to control the passive dispersion of OSW to prevent its rapid spread from Puglia to the main Italian citrus areas. The results of this monitoring demonstrated that OSW is by now acclimatized not only in Lecce's province, but apparently in whole Apulia and its eradication is almost impossible. Anon (1975) and van den Berg & De Beer (1997) reported that *A. spiniferus* was reported to be one of the most destructive aleyrodids attacking citrus in tropical Asia. The leaves, fruit and branches of infested trees are usually covered with sooty mould and a reduction in tree vigour and production usually occur. In Italy and in the EPPO, (Porcelli, 2008) reported it in Apulia when its presence seemed to be limited to this region alone. At the end of 2009,Coffi et al, 2013 reported that the pest was found to infest 68 of the 97 municipalities of the Lecce district, with a various degree in infestation intensity. One year later, A. spiniferus infested 88 municipalities but was still absent in Diso, Guagnano, Melendugno, Novoli, Salice Salentino, Squinzano, Trepuzzi, Uggiano la Chiesa and Veglie. These nine non-infested municipalities are located along the Brindisi-Taranto districts border, on the Adriatic coast, at the north edge of the infested area. During 2011, the pest spread into the villages alongside the Adriatic coast, only Diso and Melendugno being pest-free, apparently. Inspections in April 2011 showed pest outbreaks in San Pancrazio Salentino, a village in the Brindisi district where *A. spiniferus* was recorded for the first time on Citrus limon in a private garden.

The adults of this species are weak fliers, able to fly under wind for short distances, so this cannot be considered long-range mode dispersion (Meyerdink *et al.,* 1979). For which the diffusion in a short distance, can be caused by the wind and habit of adults" Take a pass” on clothes, car or other means of transportation. The spread long distance is given, probably through nymphal or pupal stages of the pest on infested plant materials.

Aleurocanthus spp. have been intercepted on the leaves of infested host plants moving in international trade (Anonymous,1988). Through the present study, it can be presumed that *A. spiniferus* was introduced in a single event. The introduction of this whitefly species had several consequences:

1. A strong disturbance of natural and artificial environments by an increase in the use of pesticides in economic and recreation areas and in private gardens. Moreover, it is spreading on the wild flora that is now the main pest reservoir in Italy.

2. Plants are damaged by sap-sucking and by excreting copious amounts of honeydew that coats leaf and fruit surfaces. Sooty mould develops quickly on this honeydew as flakes that can be peeled off from the leaves. The plant blackening reduces respiration and photosynthesis, giving low quality, small and permanently black-stained products, resulting in plants and fruits non traceable (Figures 7 and 8).

3. In order to dispose blackened vegetation, growers severely prune infested branches in their orchards eventually. This measure resolves into re-sprouting of abundant twigs and, thus, into new intense infestations.

4. Weakening of severely infested trees results in discoloration of leaves and defoliation, die back of branches and twigs, disfigured plants, deformed and dropping fruits Citrus production by over 45-50%, if not properly managed (Bambara, 2011). Chemical control alone is both expensive and ineffective (Gyeltshen *et al.,* 2010) and after overwintering as nymphs and puparia, infestations grow considerably in spring-summer starting from neglected orchards and wild flora. The introduction of such useful parasitoids, in particular *E. smithi*, in a Classical Biological Control (CBC) program seems to be the only effective measure to control *A. spiniferus* in Italy. Moreover, CBC is prevented in Europe by the Habitats Directive 92/43/EEC and in Italy by transposed regulations (DPR 357/1997, DPR 120/2003), so find out effective indigenous natural enemies which may be useful in an ABC program, the only available chance at the moment. In the present situation, the only option for pest control is to find indigenous effective natural enemies by exposing infested Citrus plants as baits in semi-natural environments.

The spread of the insect in Italy remains almost unchallenged by enemies Natural natives, because they were not found until now specific natural enemis in Puglia, only predators such as beetles nonspecific *Clitostethus arcuatus* and *Oenopia* *conglobata* which by themselves are unable to control pests efficiently more intense.coffe et al,2011,2013.

The results obtained from the tests of predation are in line with the bibliography relative to the predator produced in italy. Studies in Sicily on *Dialeurodes citri* (Liotta, 1981) show that the larva during its development can attack about 300 eggs, while the adult who lives about 2 months, can stick up to 1500 eggs. The evidence of predation made in this work show that the larval Beetle requires about 250-300 *A. spiniferus* eggs to achieve the adult stage, while adults preying on average 25-30 eggs a day.

As part of the prsent we have shown that both species of beetles studied manage to complete their life cycle preying *A. spiniferus* exclusively, while further biological parameters will be the next, more detailed studies.

We can consider *Clitostethus arcuatus* and *Oenopia conglobata* which factors biological containment. Specifically then you could achieve Mass breeding of the two predators followed by their release in environments infested: the two spider beetles have been shown to be active predators of *A.spiniferus* and be able to complete their development cycle on plants Citrus infested with OSW. Although their effectiveness remains of predation contained in respect of particularly heavy infestations, a release in mass in citrus groves with initial infestation degree could bring down the parasitic pressure, while more intensely infested citrus could act in a synergistic way with other operations in the framework of control plans integrated.

It remains however to be considered that the introduction of useful parasitoids, in particular Encarsia smithi (Silvestri), in a cross-border program It might be the only really effective measure to control *Aleurocanthus spiniferus* in Italy.

The mode of presentation of an artificial diet is important in determining its acceptance by a predator (Cohen & Staten 1993; Grenier *et al.,* 1994 and Cohen 2004). Important issues in diet presentation include phago-stimulants, texture, liquid or semi-solid state of the ingredients, and methods of containment. One of the most important factors limiting the use of coccinellid beetles in the biological control of certain insect pests is the difficulty experienced in rearing sufficient quantities. The standard method of rearing them on suitable hosts is often impracticable because of their restrictive diets. For this reason, considerable time and effort was devoted to the development of a satisfactory and economic technique for the rearing of these predators on artificial media.

The most cost-effective means of rearing predators most likely involves the creation of artificial diets that eliminate the need to use any insect components; research leading in this direction is ongoing (Singh 1982; Thompson 1999; Thompson and Hagen 1999). Using a deletion and addition approach, Arijs and De Clercq (2001b) found that egg yolk and beef liver were important components, whereas ground beef, ascorbic acid and sucrose were minor components in an artificial diet that supported the development of an anthocorid, *O. laevigatus*. Excessive amounts of any additive in the diet caused the production of undersized adults.

The next level of progression in cost-effective rearing of predators may involve the utilization of an artificial diet that obviates the use of prey. There are three classifications of artificial diets, based on the degree that the constituents are clearly defined. These include holidic diets, in which all constituents are known in chemical (i.e., molecular) structure, meridic diets, in which most of the constituents are known chemically, and oligidic diets, in which few of the constituents are known chemically (Dougherty 1959). However, the distinction between these three classi- fications has not always been clear. Another classification system has separated artificial diets based on whether they contained insect components (i.e., tissues, hemolymph, cells, protein, amino acids, etc.) or not (Grenier and De Clercq 2003). Artificial diets containing insect components are useful when predators require certain growth factors, feeding stimulants and other chemical cues that are typically found in arthropod prey (De Clercq 2004)

The chances of developing an effective artificial diet and automated system for mass rearing are probably much greater for a generalist than a specialist predator (Cohen et al. 1999). Unfortunately, generalist (polyphagous) predators have come under tremendous scrutiny because of concerns of the potential adverse effects they might pose to the environment (Strong and Pemberton 2001; van Lenteren et al. 2003). Accumulating evidence suggests that certain generalist, exotic species have the potential to out-compete native species, leading to localized extirpation of some populations (De Clercq 2002).

The multicolored Asian lady beetle, *Harmonia axyridis* (Pallas), was able to complete its development on *E. kuehniella* eggs (Specty et al. 2003). *E. kuehniella* eggs were richer in protein (amino acids) and lipids, whereas, natural prey was slightly richer in carbohydrates (glycogen). Interestingly, mortality during the larval stage decreased, body weight of emerged adults increased, and fecundity increased when *E. kuehniella* eggs, rather than aphids, were used as prey. The authors concluded that *H. axyridis* has considerable plasticity to adapt to different food sources and compensate accordingly.

Parrella *et al.,* (2015) in November 2014, found adults of *H. axyridis*. in a country house located in the San Leucio del Sannio (BN) municipality, in Southern Italy. The colour form found consisted of 96% *H. axyridis* f. succinea, 2.7% *H. axyridis* f. spectabilis and 1.3% *H. ayridis* f. conspiqua (n=75). This represent the first report of *H. ax*iridis from Southern Italy.

Sun and Fang (1999) reviewed the highest egg productivity (3000 or more per female) achieved by feeding adults on *Trichogramma* sp., pupae improved by the addition of a mixture of 0.1 % olive oil and 5% cane sugar or on *Trichogramma* adults alone. Singh and Singh (1994) compiled life fecundity tables for females of *C. septempunctata* preying on *L. Erysimi* and found that the net reproductive rate and mean length of a generation under laboratory and field conditions were 95.88 and 54.18, and 28.88 and 28.68 days, respectively.

Generalist predators are known to greatly contribute to biological control of many agricultural pests in the word [Symondson et al, 2002]. In the last five years, studies have documented the biology and effectiveness of the zoophytophagous predatory *Macrolophus pygmaeus* Rambur (Hemiptera, Miridae) to control various crop pests [Perez-Hedo et al, 2015[1], Perez-Hedo M et al, 2015[2]] Those predatory mirids are efficient natural enemies for controlling whiteflies, thrips, aphids, mites and lepidopteran pests [Alomar et al, 2002– Perdikis et al,2011].

Perdikis *et al.* (1999) studied the effect of temperature and photoperiod on the rate of predation of nymphs and adults of the predator, *M. Pygmaeus* using *Myzus* *persicae* as prey feeding on eggplant and pepper plants. The rate of predation increased with increase of temperature. Predation rate was affected by photoperiod on pepper, but not on eggplant. Females and fifth instar nymphs were the most voracious stages, followed by third and fourth instar nymphs and males. First and second instar nymphs consumed fewer aphids. Predation rate was higher on leaves of pepper than eggplant, especially at 30°C.

Sònia et al,2012 examined he effect of the host on the interactions between the predator *Macrolophus pygmaeus* and the parasitoid *Eretmocerus mundus*, natural enemies of *Bemisia tabaci*. *M. pygmaeus* preferred to consume *B. tabaci* over *E. mundus* when immature stages and adults of *B. tabaci* and *E. mundus* were offered. They consumed a larger amount of healthy *B. tabaci* nymphs and adults than of parasitised nymphs or *E. mundus* adults. The joint release of *M. pygmaeus* and *E. mundus* adults did not increase the control of the whitefly *B. tabaci*.

Lykouressis et al 2009,stueied the Predation Rates of *M.pygmaeus* on Different Densities of Eggs and Nymphal Instars of *Trialeurodes vaporariorum*. showed that the predation rates increased with prey density. The highest mean daily consumption was 94 eggs, and 56. 5, 24. 4 and 11. 8 nymphs of the 2nd, 3rd and 4th nymphal instar, These results indicate a high potential of *M. pygmaeus* as a biological control agent of the greenhouse whitefly.

Integrated pest management (IPM) is promoted by FAO and Europe (Directive 2009/128/EC) as a sustainable approach to crop protection that minimizes the use of pesticides. It is based on the combination of preventive methods and monitoring of pests and their damage, but also on the use of biological, physical, and other sustainable non-chemical methods if they provide suitable pest control. Biological control (BC) which relies on the use of living organisms (natural enemies) to reduce pest populations is a key component of IPM [Desneux et al, 2010, Urbanejaet al, 2013, Zappalà et al,2013]. It includes classical (introduction of natural enemies to a new area), augmentation (supplemental release of natural enemies), and conservation BC (habitat managed to favor natural enemies). However, biological control is not widely implemented in pest management programs, mostly due to growers' lack of knowledge on biology and ecology of both pests and their natural enemies.

Chapter 5

5. Conclusion

In the Puglia region and in the province of Lecce, the whitefly has acclimated and was spreading on the territory of Salento, infesting many cultivated plants, ornamental and spontaneous. The adaptability in that zone and the resulting diffusion, determines difficulties in controlling the pest and pose major obstacles for its eradication.The introduction of *A. spiniferus* has several consequences (Cioffi *et al.,* 2013):

1. Strong disturbance of natural and artificial environments; consequent increase in the use of plant protection products (pesticides) in economic and recreation areas and in private gardens. Also, spreading on wild flora which is the main tank of the insect.

2. The plants are damaged by insect food which subtracts SAP and produces large quantities of honeydew as flakes that can be peeled off from the leaves. The plant blackening reduces respiration and photosynthesis, giving low quality, small and permanently black-stained products, resulting in plants and fruits non tradeable.

3. Weakening of severely infested trees results in discoloration of leaves and defoliation, dieback of branches and twigs, disfigured plants, deformed and dropping fruits.

5. The pest damages and destroys the orchards severely.

6. Injuries resemble those of *A. spiniferus*: ultimately trees are stunted, cease to flower and to produce fruit, and may become so weakened that they are unable to withstand unfavorable soil or weather conditions and invasion by diseases (Russell, 1962).

Recommended *Aleurocanthus spiniferus* control method:

(1) Cutting off intensive pest branches, the orchard ventilation, timely cultivation, fertilization, enhance vigor, improve plant resistance the ability of insects.

(2) The control index for the average *A. spiniferus* each leaf has 2 heads, namely prevention. When the 1st instar larvae and the 2nd instar larvae accounted (80%, and 20%) is the optimum control period. Can choose 40% dimethoate, malathion 50%. 50% phoxim EC 1000 times liquid, 25% buprofezin EC 1000 times liquid, 2.5% ~ 2000 EC 1500 times liquid of Uranus. The safety interval corresponding to 10 days, 10 days, 5 days, 14 days and 6 days, respectively.

(3) A lot of black fly species of natural enemies, including parasitoids and predatory ladybugs, parasitic fungi, should pay attention to the protection.

(4) Nowadays, these pests are fully integrated in agro-ecosystems and are successfully controlled by IPM programs based on the use of natural enemies, particularly generalist predators.

The development of artificial diets for natural enemies offers the potential for rearing these beneficial insects in the absence of their host or prey, as well as the latter's food. The artificial rearing procedure may simplify the mass production of entomophagous insects and facilitate experimental studies of their biology, physiology and behaviour.Pest control studies often require artificial rearing systems to obtain enough healthy individuals of the target pest to allow studies of the pest itself. Artificial rearing systems are also necessary for rearing biological control agents including parasitoids and predators (van Lenteren and Tommasini 2003), as well as sterile insects (Knipling 1966).

Studies with several species fed artificial media showed that parasitoids and predators have no distinct qualitative nutrient requirements, which are similar to those of other insects. Nutrient balance is however, critical and optimal growth is achieved with high-protein diet (Thompson, 1999). Considerable importance must be given to the quality control of the beneficial insects produced in vitro in terms of a number of characteristics including longevity, fecundity and host/prey searching capacity under field conditions (Grenier & De Clercq, 2003). Other quality parameters (including fecundity and total aminoacid larval body content) were however lower in artificially than in naturally reared natural enemies .

Insects that are reared on artificial diets are used in many programs: as agents of biological control and sterile insect technologies (Knipling, 1979), One of their most important uses is in research on virtually all areas of entomology and of other biological sciences.The accomplishments in development, improvement, and application of artificial diets have come from direct efforts to suit the needs of insects by studying the target insects and, less directly, from application of a knowledge base of various aspects of food sciences and their related disciplines (nutrition, microbiology,and biochemistry, for example). Although the accomplishments associated with applications of insect diets are noteworthy, with a better understanding of insect diets, progress in entomology could be much accelerated and amplified

In the present study, although the ability of *M. pygmaeus* to complete its development feeding on *A. spiniferus* was not checked, we showed that at least in the short term, *A. spiniferus* could be a suitable prey for *M. pygmaeus*. The predatory efficiency of adults/5th nymph was evaluated because they make use of new prey resources.Therefore, these results cannot be generalized without further study. Commercially available *M. pygmaeus* are primarily sold to control whiteflies and *Tuta absoluta* in tomato crops, although prospects for its use as a biocontrol agent of *A. spiniferus* in other crops could be considered. In this context, our results are relevant and highlight the importance of considering the effect of mirid predation on A. spiniferus. We also demonstrate the efficiency of *M. pygmaeus* for controlling A. *spiniferus*, especially when it is used in augmentative biological control. *M. pygmaeus* predation on *A. spiniferus* is described here for the first time. However, the observation that *M. pygmaeus* feeds on *A. spiniferus* in the laboratory indicates some interesting lines of research. If this were the case then this mirid bug could positively contribute to the biological control of *A. spiniferus*.

Chapter 6

6. References

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Annex (1): Sampling sites and dates, host plants and status of OSW in Bari and Taranto city, 2012-2014

N°	Date	Place	Host plant	Results
		PROVINCE OF BARI		
1-7	23/09/2013 23/09/2013 23/09/2013 23/09/2013 23/09/2013 24/09/2013 16/04/2014	Bari Amendola Street corner with Postiglione Street; Padre Pio Street; Garden between Viale della Repubblica Luigi Galvani street; Orabona Street,university campus; Amendola Street 207, bed & breakfast Executive l'Orangerie; Nizza Street; Corso Alcide de Gasperi (garden).	*Parthenocissus tricuspidata, Rosa* sp., *Citrus* sp., *Vitis vinifera,* CV *Regina*	Positive
8	24/09/2013	Caccuri Street (urban garden near Hotel Nicholaus).	*Parthenocissus tricuspidata*	Negative
9	26/04/2013 24/09/2013 10/06/2014	Bitritto Circumvallation Nicola Bellomo Street.	*Parthenocissus tricuspidata* *Rosa* sp. *Citrus* sp.	Negative
10	24/09/2013 10/06/2014	Bari Giovanni Gentile Street Near Carbur drink shop N.73 Autocarrozzeria Meselli N.58	*Vitis vinifera*	Negative

11	12/08/2012 24/09/2013 10/06/2014	Casamassima, SS 100, Noicattaro street 2, near Auchan Commercial Centre Casamassima, City garden	*Parthenocissus tricuspidata*	Negative
12	24/09/2013 10/06/2014	Noicattaro Corso Roma, City garden	*Citrus* sp. *Vitis vinifera*	Negative
13	24/09/2013 10/06/2014	Noicattaro - Rutigliano, SP to Rutigliano Garden, Noicattaro, near Esposizione Settanni S.r.l. Uffici Officina 634 Km 72	*Vitis vinifera*	Negative
14	24/09/2013 10/06/2014	Polignano San Vito San Giovanni F.lli Spada S.n.c. Gas station SS16, KM 835 Ripagnola Di Teofilo G. C. Gas station SS 16 Km. 829.257	*Parthenocissus tricuspidata, Citrus* sp.	Negative
15	25/09/2013 18/06/2014	Noicattaro Centro storico Corso Roma	*Citrus* spp	Negative
16	25/09/2013 18/06/2014	Noicattaro SP 57, Gas station Q8 km 1,250	*Parthenocissus tricuspidata, Citrus* spp	Negative
17	25/09/2013 18/06/2014	Mola di Bari - Polignano a Mare SS 16 Eni & Total Erg Gas station km 829.257	*Parthenocissus tricuspidata, Citrus* spp	Negative
18	26/09/2013 27/06/2014	Bari San Paolo, SP 73 Viale Europa near “Suditalia Gas” Gas Station 18/b	*Parthenocissus tricuspidata, Rose, Citrus*	Negative
19	26/09/2013 27/06/2014	Bari Palese, city garden Nisio Girolamo Street	*Parthenocissus tricuspidata,*	Negative

		Duca D'Aosta Street	*Rose, Citrus* spp	
20	26/09/2013 27/06/2014	SS 16 Bis Santo Spirito, Giovinazzo Complanare Sud, Gas station ENI km 787,220	*Citrus* spp	Negative
21	28/04/2014	Bari Torre a mare, SS16, Gas stations Q8 km 811.400 Esso SS 16	*Ficus* spp, *Parthenocissus tricuspidata*	Negative
22	28/04/2014	Noicattaro,Torre a mare street N.68 private house garden	*Citrus limon, Vitis vinifera*	Positive
23	28/04/2014	Cozze, City garden	*Parthenocissus tricuspidata*	Negative
24	07/05/2014	Polignano a Mare SS16 San Vito San Gionanni, Gas Station Q8km 833,454	*Pittosporum tobira*	Negative
25	28/04/2014	Polignano a Mare-Monopoli SS16 Gas station Total Erg km 839.950	*Pittosporum tobira*	Negative
26	08/04/2014	Gioia del Colle, City garden	*Citrus, Prunus laurocerasus*	Negative
27	12/07/2012 17/04/2013 09/05/2014	Modugno, City garden	*Citrus* spp	Negative
28	12/07/2012 17/04/2013 09/05/2014	Bitonto, City garden	*Citrus* spp	Negative
29	12/07/2012 17/04/2013	Giovinazzo, City garden	*Citrus* spp	Negative

	09/05/2014			
30	12/07/2012 17/04/2013 09/05/2014	Molfetta, City garden	*Citrus, Prunus laurocerasus*	Negative
31	12/07/2012 17/04/2013 09/05/2014	Terlizzi, City garden	*Citrus, Prunus laurocerasus*	Negative
32	12/07/2012 17/04/2013 09/05/2014	Ruvo di Puglia, City garden	*Citrus* spp	Negative
33	26/04/2013 12/05/2014	Bitritto, City garden	*Citrus* spp	Negative
34	26/04/2013 12/05/2014	Binetto, City garden	*Citrus* spp	Negative
35	26/04/2013 12/05/2014	Bitetto, City garden	*Rose, Citrus* spp	Negative
36	26/04/2013 12/05/2014	Palo del Colle, City garden	*Citrus* spp	Negative
37	26/04/2013 12/05/2014	Toritto, City garden	*Citrus* spp	Negative
38	26/04/2013 12/05/2014	Grumo Appula, City garden	*Rose, Citrus* spp	Negative
39	06/05/2013 15/05/2014	Sannicandro di Bari, City garden	*Citrus* spp	Negative
40	20/04/2012 06/05/2013 15/05/2014	Adelfia, City garden	*Citrus* spp, *Vitis vinifera*	Negative
41	20/04/2012 06/05/2013 15/05/2014	Valenzano, City garden	*Citrus* spp	Negative

42	21/09/2012 06/05/2013 15/05/2014	Capurso, City garden	*Citrus* spp,*Vitis vinifera*	Negative
43	21/09/2012 06/05/2013 15/05/2014	Cellamare, City garden	*Citrus* spp	Negative
44	21/09/2012 06/05/2013 15/05/2014	Triggiano, City garden	*Ficus* spp, *Parthenocissus tricuspidata, Vitis vinifera*	Negative
45	21/09/2012 16/05/2013 16/05/2014	Conversano, City garden	*Citrus* spp	Negative
46	12/08/2012 16/05/2013 16/05/2014	Turi, City garden	*Citrus* spp	Negative
47	21/09/2012 16/05/2013 16/05/2014	Putignano, City garden	*Rose, Citrus spp*	Negative
48	21/09/2012 15/05/2012 16/05/2014	Noci, City garden	*Citrus* spp	Negative
49	12/08/2012 16/05/2013 16/05/2014	Castellana Grotte, City garden	*Rose, Citrus spp*	Negative
50	21/09/2012 15/05/20112 16/05/2014	Alberobello, City garden	*Citrus* spp	Negative
51	21/09/2012 15/05/2012 16/05/2014	Monopoli, City garden	*Citrus* spp	Negative

52	15/05/2012 16/05/2014	Locorotondo, City garden	*Rose, Citrus spp*	Negative
		PROVINCE OF TARANTO		
53	27/09/2013	Taranto, Ciro Giovinazzi Street, City garden	*Citrus* spp *Parthenocissus tricuspidata* *Rose, Citrus* spp	Positive
54	27/09/2013	Taranto, City garden Pantano		Negative
55	20/04/2012 27/09/2013 08/04/2014	Massafra, City garden Pezzata rossa Patemisco Chiatona Albanello Dicolillo Mazzarelle Ciula	*Parthenocissus tricuspidata, Rose, Citrus* spp, *Vitis vinifera*	Negative
56	20/04/2012 13/06/2012 27/09/2013 08/04/2014	Palagiano, City garden Lupini San Marco Conocchiella Lenne Marchiotta Galliano Lama d'erchie Conca d'oro Monticello	*Parthenocissus tricuspidata, Rose, Citrus* spp	Negative
57	20/04/2012 13/06/2012 28/09/2013	Palagianello, City garden Parco di stalla Difesella	*Parthenocissus tricuspidata,*	Negative

	08/04/2014	Lenne	*Rose, Citrus* spp	
58	05/07/2012 28/09/2013 22/04/2014	Castellaneta, City garden Borgo Perrone Le Ferre Salesiano Gaudella Serenella Terzo dieci Scapati	*Parthenocissus tricuspidata, Rose, Citrus*spp, *Vitis*	Negative
59	05/07/2012 28/09/2013 22/04/2014	Ginosa marina, City garden Magliati Girifalco Stornara Pantano Montedoro Lama di pozzo	*Parthenocissus tricuspidata, Rose, Citrus* spp	Negative
60	05/07/2012 27/09/2013 22/04/2014	Statte, City garden Accetta Grande Gravinella Accetta Piccola	*Parthenocissus tricuspidata, Rose, Citrus* spp	Negative
61	05/07/2012 13/09/2012 21/05/2014	San Giorgio Ionico, City garden	*Parthenocissus tricuspidata,*	Negative
62	24/07/2014	Monteparano, City garden	*Citrus*	Positive
63	13/09/2012 24/07/2014	Fragagnano, City garden	*Citrus*	Positive
64	13/06/2012 13/09/2012 21/05/2014	Manduria, City garden	*Citrus, Vitis vinifera*	Positive
65	13/06/2012 13/09/2012	Sava, City garden	*Citrus, Vitis vinifera*	Positive

	21/05/2014			
66	13/09/2012 21/05/2014	Avetrana, City garden	*Citrus*	Positive
67	04/04/2013 21/07/2014	Martina Franca, City garden	*Citrus*	Negative
68	13/06/2012 03/06/2013 21/07/2014	Crispiano, City garden	*Citrus*	Negative
69	03/06/2013 21/07/2014	Montemesola, City garden	*Rose,*	Negative
70	13/06/2012 03/06/2013 21/07/2014	Grottaglie, City garden	*Citrus, Vitis vinifera*	Negative
71	03/06/2013 21/07/2014	Monteiasi, City garden	*Citrus*	Negative
72	24/06/2013 24/07/2014	San Marzano di San Giuseppe, City garden	*Parthenocissus tricuspidata Citrus, Vitis vinifera*	Negative
73	13/06/2012 24/06/2013 24/07/2014	Carosino, City garden	*Citrus*	Negative
74	24/06/2013 24/07/2014	Rocca Forzata, City garden	*Citrus*	Negative
75	04/04/2013 24/06/2013 24/07/2014	Faggiano, City garden	*Citrus*	Negative
76	13/09/2012 25/07/2014	Leporano, City garden	*Rose, Citrus*	Negative
77	04/04/2013 25/07/2014	Pulsano, City garden	*Rose, Citrus, Vitis vinifera*	Negative
78	04/04/2013	Lizzano, City garden	*Citrus*	Negative

	25/07/2014			
79	04/04/2013 25/07/2014	Torricella, City garden	*Citrus*	Negative
80	04/04/2013 25/07/2014	Maruggio, City garden	*Citrus*	Negative
		PROVINCE OF BRINDISI		
81	12/08/2012 13/06/2013 21/05/2014	Brindisi, City garden	*Citrus*	Positive
82	30/04/2012 12/08/2012 09/04/2013 21/05/2014	Torchiarolo, City garden	*Citrus*	Positive
83	28/04/2014 12/08/2012 17/07/3014	Fasano, City garden	*Citrus*	Negative
84	28/04/2014 12/08/2012 17/07/3014	Ostuni, City garden	*Citrus*	Negative
85	13/06/2013 17/07/3014	Carovigno, City garden	*Parthenocissus tricuspidata, Citrus*	Negative
86	13/06/2013 17/07/3014	San Vito dei Normanni, City garden	*Citrus*	Negative
87	30/04/2012 12/08/2012 09/04/2013 13/06/2013 17/07/3014	Mesagne, City garden	*Citrus*	Negative
88	30/04/2012 12/08/2012	Torre Santa Susanna, City garden	*Citrus, Vitis vinifera*	Negative

	09/04/2013 13/06/2013 17/07/3014			
89	30/04/2012 12/08/2012 09/04/2013 17/07/3014	Erchie, City garden	*Citrus, Vitis vinifera*	Negative
90	13/06/2013 06/06/2014	Cisternino, City garden	*Citrus*	Negative
91	13/06/2013 06/06/2014	Ceglie Messapica, City garden	*Citrus*	Negative
92	06/06/2014	Villa Castelli, City garden	*Citrus*	Negative
93	13/09/2012 04/04/2013 06/06/2014	Francavilla Fontana, City garden	*Citrus*	Negative
94	30/04/2012 12/08/2012 09/04/2013 06/06/2014	Oria, City garden	*Citrus, Vitis vinifera*	Negative
95	13/06/2013 06/06/2014	Latiano, City garden	*Citrus, Vitis vinifera*	Negative
96	06/06/2014	San Michele Salentino, City garden	*Citrus*	Negative
97	09/04/2013 12/08/2012	San Pietro Vernotico, City garden	*Citrus*	Negative