Biodiversity Journal, 2015, 6 (1): 147–160 **MONOGRAPH**

Pest management of citrus fruits in Sicily (Italy) through interventions of biological control. The example of the biofactory of Ramacca, Catania

Giuseppe Greco

Ente di Sviluppo Agricolo della Regione Siciliana (E.S.A.), via Libertà 203, 90143 Palermo, Italy

ABSTRACT Since 2007, in Sicily, plant health protection against citrus mealybugs is taking place through the Biofactory of Ramacca, in the Plain of Catania, a property of the Institute for Agricultural Development of the Sicilian Region (i.e. Ente per lo Sviluppo Agricolo, E.S.A.). The Biofactory is unique being aimed to produce industrial quantities of auxiliary insects and is a center of European interest because it is fully organized to provide means of biological fight imposed by the Directive 128/2009/EC, which requires, from 1 January 2014, farms to comply with the application of general principles of integrated pest management. In this paper we examine structural features of the Biofactory, breeding techniques empoyed and results obtained in the period 2007–2013, which allowed many companies, from 200 to 360 (i.e. 20% –35% of the regional surface operating in organic citrus production) to be able to employ biological weapons against pest insects. We analyze dynamics and results of production deriving from the approval and adoption, by the owner (E.S.A.), of a new "discipline" that governs the assignment of insects to farmers at a very low price to balance E.S.A.'s purposes, which is both to ensure adequate performance in order to pursue institutional support to agriculture and, considering the Insitute's economic nature, to partially cover the production costs incurred to ensure the service. The continuity of the project is assured by the ongoing program for the period 2013–2020 with an enlargement of the array of entomological production aimed at intercepting the needs of new productions (i.e. greenhouse horticulture, vines, ornamental and fruit trees).

KEY WORDS pest management; biological control; Biofactory; Ramacca; Sicily.

Received 05.07.2014; accepted 12.10.2014; printed 30.03.2015
Proceedings of the 2nd International Congress "Speciation and Taxonomy", May 16th-18th 2014, Cefalù-Castelbuono (Italy)

INTRODUCTION

A biofactory (or commercial insectary) is a structure in which takes place the breeding of arthropods on an industrial scale, aimed at the production of living organisms to be released in large amounts into the environment in the context of techniques of biological control and integrated pest management. On the contrary, the insectary is a breeding realized for scientific purposes.

The multinationals of chemistry have never seen welcome the birth of biofactories, because the organic product stands as alternative to the use of pesticides (Tremblay, 1988; Pollini et al., 1988; Goidànich et al., 1990; Flint, 1991; Grafton-Cardwell & Reagan, 1995; Pollini, 1998; Ferrari et al., 2000, 2006; Masutti & Zangheri, 2001; Muccinelli, 2006; Penny & Cranston, 2006).

There are reports of a first biofactory already in 1916 in Santa Paula, California, the “Limoneira Company”. In 1931, there were 16 and produced especially insects antagonist to citrus mealybugs like the coccinellid *Cryptolaemus montrouzieri* that is bred and successfully launched today.

In Northern Europe biofactories are used for biological control in greenhouses: here the chemical control had shown its serious limitations in the effectiveness of and compatibility with healthy products. In fact, the glass or plastic covers are an insurmountable physical barrier for antagonists of harmful species, warming accelerates the development of both plants and pests, the collection of the products can not be reconciled with respect to the "waiting period" fixed by law between chemical treatment and collection and, not least, greenhouses turn out to be "gas chambers" for the farmers who work therein.

A careless use of chemical products in agriculture with the aim to maximize the production has led over the years to a number of disorders that have resulted in considerable damage to the environment and to humans. Many chemicals have been banned and the defense of the plants has been oriented to the use of alternative methods equally effective and safeguarding the ecosystems (De Bach et al., 1969; Viggiani, 1977; Chiri, 1987; Walde et al., 1989; Celli et al., 1991; Hoffmann & Frodshan, 1993; Luck et al., 1996; Murdoch et al., 1996; Ferrari, et al., 2000; Vacante & Benuzzi, 2004; Sorribas et al., 2008, 2010; Tena & Garcia-Mari, 2011).

The first biofactories in Europe born in England and Holland around 1960 and, since then, have always grown both in number and in quantity of species bred and used. Today in Europe there are 26 biofactories with more than 30 species raised and excellent qualitative-quantitative standards.

In Italy there are only two biofactories: the first (in order of construction) is in Cesena (1987/90) while the second is in Sicily, in the Plain of Catania (Figs. 1, 2) in the territory of Ramacca (2001/03) (Greco 2014a, b). The latter is mainly distinguished by the quality and quantity of its products supplied aiming more at the diffusion of breeding techniques rather than for commercial purposes. Both biofactories serve an agricultural area which is considerably increasing in size, and achieve agricultural productions with the least possible impact on the territory, sustainable for the planet, whereas in other parts of the world, biological control has totally replaced chemical poisons.

Yet here, in the Mediterranean, people are not deeply aware of the benefits of this resource and the many solutions it offers, but the products of a biofactory are going to become even more relevant in the light of Directive 128/2009 / EC establishing a framework for Community action to achieve a sustainable use of pesticides.

This Directive was transposed into Italian law by Legislative Decree 150 of 14 August 2012. Since 1 January 2014, professional users of phytosanitary products (art. 19) should apply the general principles of integrated pest management required

Figure 1. Biofactory of Ramacca, Catania, Italy, Institute for Agricultural Development of the Sicilian Region (E.S.A.).

Figure 2. Biofactory of Ramacca, Catania, Italy: biofactory corridor.

among which is reported, as technical and fundamental element, the use of biological means of struggle.

EXPERIENCE IN SICILY, AT THE CENTER OF THE MEDITERRANEAN: THE BIOFACTORY OF RAMACCA

In 1996 the Sicilian Region has commissioned the Institute for Agricultural Development (i.e., Ente per lo Sviluppo Agricolo, E.S.A.) to study the possibility of implementing attive interventions of biological control. From that date until today E.S.A. carried out:

1) a preliminary plan for measures of biological control of *Ceratitis capitata* (Mediterranean fruit fly) at regional scale, prepared in collaboration with the FAO / IAEA Agriculture and Biotechnology Laboratory.

2) the planning of a biofactory alternative to the first one, to be built in Ramacca (Catania), aimed at the production of 3 species of insects beneficial to citrus cultivation (*Aphytis melinus*, *Cryptolaemus montrouzerii*, *Leptomastix dactylopii*) and 1 insect to be employed in horticulture. *Digliphus isaea* Walker, 1838 (Hymenoptera: Eulophidae).

Actually, it was funded and implemented only the second project in which the biofactory of Ramacca is designed to be a flexible pole of production of material (insects) to be used in agriculture for most programs of biological or integrated control. For its start-up phase of production, have been considered, as reference, those crops that, more than others, are susceptible to these kinds of initiatives for their technical and economic characteristics: citrus and protected horticulturals. Therefore the biofarm has been designed and equipped for the production of:

a) 3 insect species beneficial to biological control programs for citrus cultivation (*Aphytis melinus*, *Criptolaemus montrouzerii*, *Leptomastix dactylopii*);

b) 1 insect used for integrated pest management of vegetables and flowers grown under cover (*Diglyphus isaea*).

The factory is located in the territory of Ramacca (Catania), Margherito district, on a total area of approximately 3.5 hectares that can be potentially increased and improved in case of changed conditions of the market.

The biofactory is composed of:

a) 1 shed of 2,500 sqm (72 ml, 10.00 ml x 34,30) which houses cells in a controlled and conditioned environment;

b) 6 greenhouses, each of 100 sqm ca. (10.00 ml x 10.00 ml), five of which are used for the production of *Diglyphus isaea* and one for *Lyriomiza* (guest of *Diglyphus*), this latter room is placed at a safe distance to avoid contamination between competitors since both species are raised in purity.

The 6 greenhouses are heated, to prolong the production season even in the coldest months (January and February), and equipped with an adequate irrigation system to allow the cultivation of bean plants in pots placed on anti-algae cloths;

c) 1 office building of 350 sqm (ml 34.30 ml x 10.00 ml).

The shed is composed of 36 rooms including cells, work rooms, service corridors, warehouse, workshop, toilets and trasformer room, central cooling and boiler. Cold storage and processing rooms are 28, divided as follows:

9 for *Aphytis melinus*;
6 for *Criptolaemus montrouzerii*
4 for *Leptomastix dactylopii*
9 in common for *Criptolaemus montrouzerii* and *Leptomastix dactylopii*.

BREEDING TECHNIQUES OF INSECT PRODUCTS IN BIOFACTORY

Aphytis melinus De Bach, 1959
Hymenoptera Aphelinidae

Aphytis melinus (Figs. 3, 4) is a parasitoid of *Aonidiella aurantii* Maskell, 1879 (Rhynchota Homoptera Diaspididae), or California red scale, a major pest of citrus, but it can also parasitize other species such Diaspididae *Aonidiella citrina* (Coquillett, 1891) and *Aspidiotus nerii* Bouché, 1833 (Flanders, 1953; De Bach & Argyriou, 1967; Abdelrahman, 1974; Rosen & Eliraz, 1978; Rosen et al., 1979; Luck et al., 1982; Orphanides, 1984; Yu et al., 1986; Opp & Luck, 1986; Reeve, 1987; Yu & Luck, 1988; Rodrigo & García-Marí, 1990,

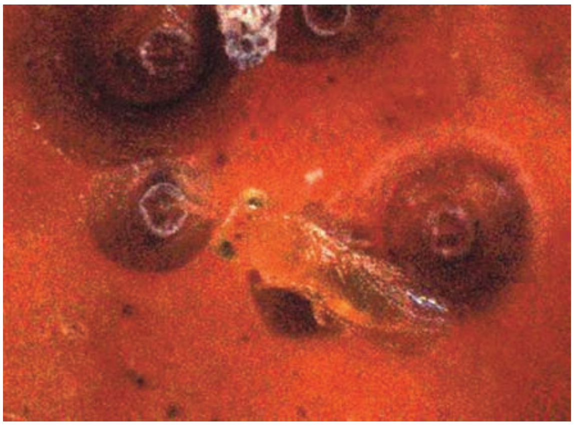

Figure 3. *Aphytis melinus*
(Photo by "Centrale Ortofrutticola of Cesena, Italy).

Figure 4. Climate cabinets with *Aspidiotus nerii* bred on pumpkins for developing of *Aphytis melinus*.

1992; Hare & Luck, 1994; Heimpel & Rosenheim, 1995; Tumminelli et al., 1996; Gottlieb et al., 1998; Pekas et al., 2003; Pasotti et al., 2004; Rodrigo et al., 2004; Pina, 2007; Pina T. & Verdú M.J., 2007; Vacas et al., 2009; Vanaclocha et al., 2009).

Agricultural use of the insect: *A. melinus is* launched at the adult stage and disperses easily in all the citrus grove, possessing excellent research skills. In citrus infected is good practice to make a winter treatment with white oil at 2-2.5%; this allows to reduce, albeit only partially, the wintering population of the cochineal. The parasitoid is launched following a pattern that includes a series of consecutive launches after the flight detection of cochineal males in late April-early May. When the plan of biological control is set up, in the first year are expected about 10-12 launches, 2/3 of which to be carried out in April-May-June until mid-July, while the remaining 3 or 4 launches take place from mid-September to throughout October. In the months of April, May and June, launches can be made every two weeks, moving on to a weekly frequency when temperatures increase. 8,000 to 12,000 parasitoids per hectare, for a total of 100 to 150,000 / ha for production season are launched. In 2-3 years the intensity of the pest is reduced so that is possible to reduce proportionally the number of lauches, limiting them exclusively to the spring-summer period. It is very important to pay attention to chemical treatments performed before and to those that will take place.

Breeding techniques and production cycle in biofactory: breeding of *Aphytis melinus* is made in climate cabinets, using the parthenogenetic strain of *Aspidiotus nerii* bred on pumpkins.

Pumpkins are kept in cells furnished with metal shelves; the environment of the cells is adjusted so as to have 13 ± 1 °C and 50 ± 5% RH; pumpkins are previously washed and disinfected.

The production process has a duration of about 60 days, breeding is carried out in two areas: one for the multiplication of the host and one for the production of the parasitoid. Even the *Aspidiotus nerii* (host) is reared in cells whose furniture is made of metal shelving with lozenges. The nymphs of *Aspidiotus* are then collected and placed in a jar before inoculating other pumpkins. The environmental conditions for the breeding of *Aspidiotus* are the following: temperature 26 ± 1 °C, RH 50% ± 5.

At the 45th day, before the spill of nymphs, 10% of pumpkins are brought in the cells for development of *Aspidiotus* for harvesting nymphs to be used for the inoculation of pumpkins, whereas the remaining 90% is iplaced in plastic bins for the production of *A. melinus.* Pumpkins are put in contact with *A. melinus* for 24 h.

The adults are taken after 24 h, blowing carbon dioxide to saturation. After inoculation, pumpkins can be placed in the two cells intended for the production dell'*A. melinus*, air-conditioned to 26 ± 1 °C and 50 ± 5% RH. After 10-15 days, *A. melinus* newborn are collected after release of carbon dioxide. Insects fall to the bottom of the cabinets and are put within cylinders where are measured volumetrically. Adults collected are packaged in trays of 10,000 or 25,000 insects containing honey as

Figure 5. *Leptomastix dactylopii*
(Photo by "Centrale Ortofrutticola of Cesena, Italy).

Figure 6. *Planococcus citri*
(Photo by "Centrale Ortofrutticola of Cesena, Italy).

feed. Packages can be stored for a few days in the refrigerator ventilated at 15 °C. The production ratio is 1: 3.

Leptomastix dactylopii Howard, 1885
Hymenoptera Encyrtidae

Parasitoid (Fig. 5). Endophagous of *Planococcus citri* Risso, 1813 (Rhynchota Homoptera Pseudococcidae) (Fig. 6) (Chandler et al., 1980; Tingle & Copland, 1988, 1989). The United States are its country of origin and its cycle in nature takes place on mealybugs, *P. ficus* Signoret, 1875, *P. vitis* Ezzat et McConnell, 1963 and, in laboratory conditions, spread also over other hosts.

Natural cycle and agricultural use of the insect: at 25 °C, and 75% humidity, the cycle of *L. dactylopii* takes about 21 days. Adults, 12 hours after the flicker, begin to mate. Females move on the pseudococcid colony seeking - measuring them by antennas - for the nymphs with appropriate shape and age where to inject the eggs (one for each victim). From each egg comes out a larva that, in 13 days, making three mutes and through four larval stages, becomes pupa, at first light in colour, then darker. After a week from 'pupation, the adult flickers. Particularly remarkable it is that the larva produces chitin and hardens the outer wall by an aeroscopic plate from which it breathes atmospheric oxygen. At the end of metamorphosis, by the chewing apparatus severs an operculum placed in anal position of the host and flickers. *L. dactylopii* is an insect yellow honey with three simple eyes. Its sizes range from 0.5 to 6 mm (11 antennomeres). Males have longer and silky antennae with 10 antennomeres, females shorter and hairless (11 antennomeres). *L. dactylopii* is marketed at the adult stage and can be used on citrus fruits in combination with *Criptolaemus montrouzieri* and on ornamental plants infested by *Planococcus citri*.

Breeding techniques and production cycle in biofactory: the production cycle of *L. dactylopii* takes place entirely in climate cabinets. The host is *P. citri* (mealybugs) which is bred on potato sprouts etiolated in areas separate from those of the parasitoid. For storage of potatoes are used cells conditioned to 5 °C and 50 +/- 5% relative humidity. The breeding cycle of the parasitoid lasts 9-10 weeks. In the first stage, are produced etiolated shoots of potato which, after 2-3 weeks, are infested with the citrus mealybug. When nymphs are ready, *L. dactylopii* is inoculated. After 20 days the adults are collected with aspirators and packed in jars of 100 individuals. Insects can be stored at 15 ° C, if well fed with appropriate diets.

Cryptolaemus montrouzieri Mulsant, 1850
Coleoptera Coccinellidae

Polyphagous predator (Hodek & Honek, 1996. Milán Vargas, 1999) that can live at the expense of several Pseudococcids or even other insects (Figs. 7, 8). The adult measures about 5-6 mm has black elytra, while the head, chest, abdomen and

Figure 7. *Cryptolaemus montrouzieri* (Photo by "Centrale Ortofrutticola of Cesena, Italy)

Figure 8. Larvae of *Cryptolaemus montruozierii* on potato sprouts infested by *Planococcus citri*.

extremities of the elytra are orange. At a constant temperature of 25 °C females live about 60 days and, during this time, lay 60 to 120 eggs.

Eggs are located close to the cottony ovisacs of the prey so that and the young larva, just shelled, can easily reach its preferred food: eggs and young nymphs of the pest.

The Coccinellidae larva goes through four stages before pupating (by attaching to a support) after which it becomes an adult. It has a waxy coating to camouflage itself onto the colonies of *P. citri*, but cannot be mistaken for its larger size and its mobility. The cycle from egg to adult lasts, at 25 °C, 35 days. It is an insect native to Australia and therefore sensible to harsh winters; it has already acclimatized in many areas of southern Italy and, in the islands, winters as an adult.

Agricultural use of the insect: *Cryptolaemus* is sold at the adult stage. On citrus fruit it is used in association with *Leptomastix dactylopii* especially in the hotbeds of infestation, which are out of control of the parasitoid. In the field, it is employed from June up to August (3 months). *Cryptolaemus* could be used also on ornamental crops in greenhouses or in potted plants; on this item, it is developing an interesting market in northern Europe.

The production cycle of *Cryptolaemus* takes place entirely in climate cabinets. The host is *P. citri* (mealybugs) which is bred in purity on etiolated shoots of potato in a separate room. As *P. citri* is used as host also by *Leptomastix*, rooms designated for *P. citri* production are used for both insects.

In particular, in a section of the biofactory, there are cells for the storage of potatoes (at 13 °C and 60% RH); and in another section, cells for the development of the tubers and, still, other cells for the development of *P. citri* (at 25 °C and RH of 60 ± 5%) that will serve to feed both the auxiliaries (*Leptomastix* and *Cryptolaemus*).

In another area of the building there are cells for development of *P. citri*, cells for collection of *Cryptolaemus* and processing rooms. The breeding cycle of predator lasts 10–13 weeks. In the first phase *P. citri* is bred in purity on etiolated sprouts of potato. In breeding cells, potatoes are made germinate in the dark for 2–3 weeks; the shoots are infested with *P. citri* and the infestation is let to develop for 3–4 weeks; finally there is the inoculum with *Cryptolaemus*. Adults, collected after 35 days with vacuum cleaners, are packaged in cans from 100 to 200 units. They are then counted volumetrically. Insects can be stored at 15 ° C, even up to a month if well fed with an appropriate diet.

MANAGEMENT BIOFACTORY

In 2006, the managing of the biofactory of Ramacca began with the finding of the head-breeding strains (*Aphytis melinus*, *Cryptolaemus montrouzieri*, *Leptomastix dactylopii* and *Diglyphus isaea*) and of intermediate entomological materials (*Aspidiotus*, *Planococcus*, *Liriomyza*, etc.) of which such insects are parasitoids or predators. As planned, entomological breeding aimed, from the beginning, at the production of *Aphytis melinus*, *Leptomastix dactylopii* and *Cryptolaemus montrouzieri*. At first it was even started a production of *Diglyphus isaea* (greenhouse parasitoid on *Liriomyza trifolii*, *L. bryoniae* and *L. huidohernsis*) then abandoned because of the uneconomic production cycle.

Until 2011 the entomological material was distributed free to farmers through peripheral companies belonging to E.S.A. (i.e. SOPAT, Offices for the Antiparasitic Fight) and to the Department of Agriculture and Forestry (SOAT, OMP).

The criteria developed by the Administrative Department of biofactory included a distribution of the product to farmers cultivating citrus, to organic or converting to organic farms, and to farms that apply and implement criteria of integrated pest management, according to a programming technique agreed with local Institutes that provide agricultural technical assistance (ESA, SOAT and the Office of Agriculture and Forestry).

The reaching of full production was expected by the third year (29 March 2009), during which it has been programmed the full activity of the building with the following annual production levels:

Aphytis melinus 67,200,000 individuals;
Cryptolaemus montrouzieri 350,000 individuals;
Leptomastix dactylopii 1,000,000 individuals;
Diglyphus isaea 1,900,000 individuals.

Data management in the period 2006-2011

During the period 2006–2011 (Fig. 9), insects have been distributed free to regional farms and other applicants who had a purpose in the public interest, including regional and national Universities,

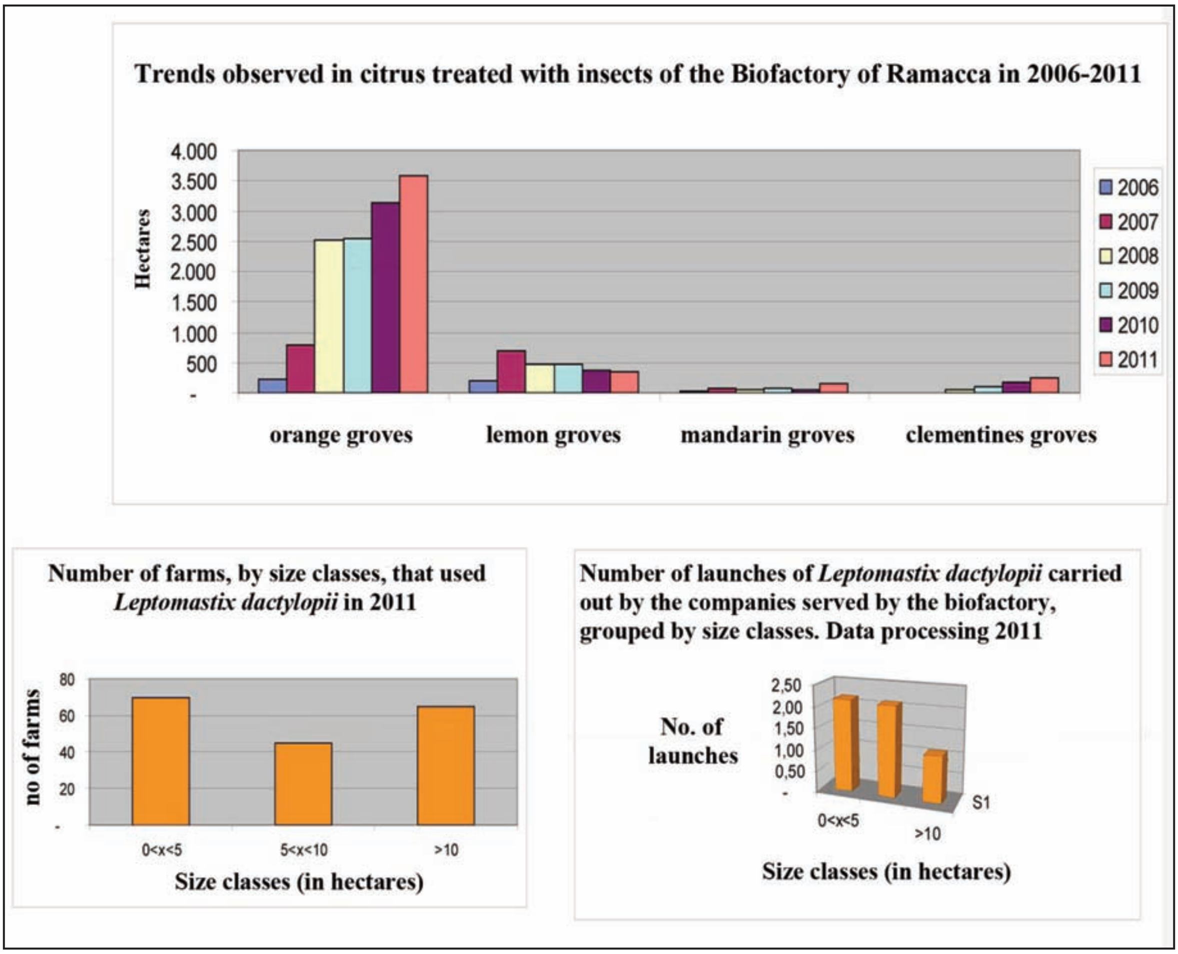

Figure 9. Data management in the period 2006-2011 (Source E.S.A.).

Regional Departments, Development Services, Institutes or Development Agencies of other Italian regions.

Maximum productions were distributed in 2010–2011, mostly to citrus farms, for a total of more than 4,300 hectares distributed in 325–355 entities. Noteworthy, as for the 2010-2011 data, there is a significant increase in production (+ 50% compared to 2010), correlated with a stabilization of the “protected” area, amounting to 4,361 hectares (-28 hectares compared to 2010); the maintenance of the substantial number of seasonal launches can be explained by a kind of loyalty of the users who, in manifesting an appreciable degree of satisfaction, show confidence in using alternative means of organic production.

Data management in the period 2012-2013

In 2011, it was suggested to apply a reduced price to Sicilian farms. This is to contribute to the costs of production that, every year, E.S.A. supports to ensure its performance. So it was approved and put into effect a new "Discipline" which regulates the sale of insects to farmers at a "price of contribution", in order to proceed, gradually, to compensate production costs. The "price of contribution", which ranks, by definition, below the values of the free market, reconciles the needs of the Istitute, which has to ensure adequate performance in providing institutional support to agriculture, with its financial nature aimed to partially cover the costs of production. This regulation does not exclude the transfer of beneficial insects also in favor of other subjects, in different places (extra-regional) and, possibly, for different purposes (agricultural as well as commercial or public). In this case, the above mentioned constraints do not apply, so that E.S.A. can set the products at different prices (to be considered net of shipping), commensurate with market values.

Application of new "Rules" recorded a drop in distribution in 2012–2013, and, during a period of 6 years of free distribution, it obviously resulted in a big change of the demand of the three species. A first effect of the new regime can be seen in the production levels of 2012-2013. In particular (Fig. 10), the amount of *Aphytis melinus*, *Leptomastix dactylopii* and *Cryptolaemus montrouzieri* - although often reached high profiles above those of feasibility - stood at levels significantly lower than those of 2011, i.e. 139–149 million, 672-1766 thousand and 233–277 thousand individuals, respectively. In 2012, production reached 119% of what expected in steady-state conditions,(i.e. +19%). Briefly, these results can be explained with a production trend that had to take into account users' requests, which resulted in a change of strategies and productive quality (when possible) that affected, for example, the extent and availability of traditional raw materials to be acquired (potatoes,

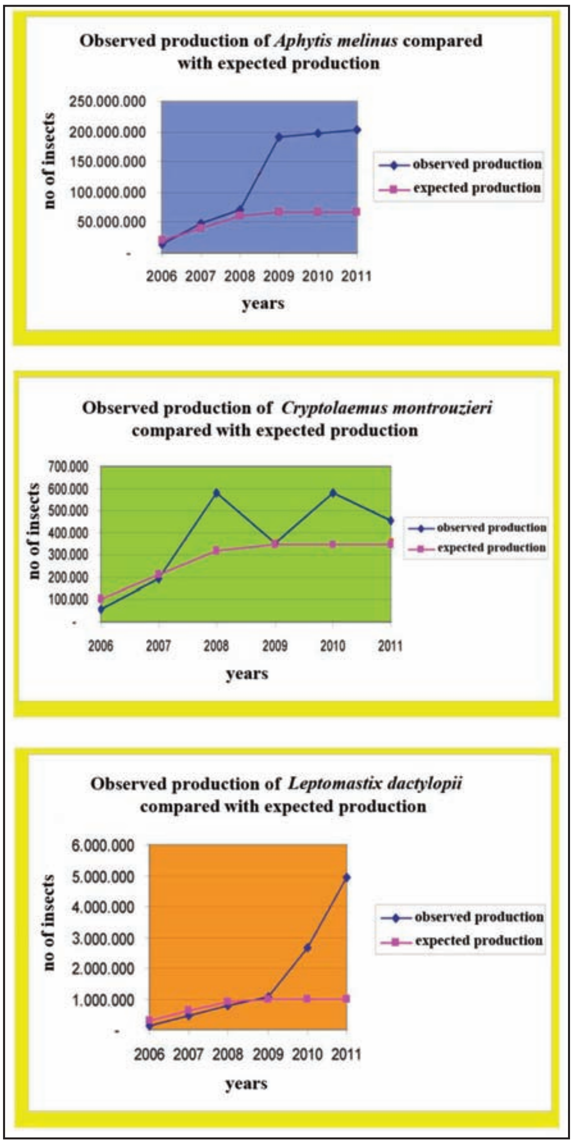

Figure 10. Development of production of *Aphytis melinus*, *Leptomastix dactylopii* and *Cryptolaemus montrouzieri* than expected feasibility (Source E.S.A.).

var. “Spunta” and “Desiree” and pumpkins var. “Butternut”). Another cause is to be found in distribution fees, which were fixed in the absence of solid experience of huge productions and, therefore, of necessary and useful market information. Finally, the price of each insect certainly influenced the users’ choise. For example the price / effectiveness or cost/utility ratio for *Aphytis melinus* was considered, by the regional users, more convenient than those fixed for *Cryptolaemus montrouzieri* and *Leptomastix dactylopii*.

Profile of user companies in 2012-2013

Quantitative aspects of each entomological entity distributed to regional farms are of course also reflected on land statistics. In fact, Users (i.e. farms), primarily engaged in citrus cultivation, were more than 200 (213 to 298), for an area of at least 2,300 Ha. Just to quantify, 2,152 Ha of orange groves, 313 ha of lemon groves, 91 Ha of mandarin groves and 51 of clementine groves took advantage from the service provided by E.S.A.

The new payment system had a negative impact not only on the lemmon groves of Syracuse: also other citrus groves suffered a regression of land extensions which reached its peak in the areas planted with orange trees. It also follows, that the biological defense against the citrus mealybug, (*P. citri*) and red scale (*A. aurantii*) by *Aphytis melinus*, *Cryptolaemus montrouzieri* and *Leptomastix dactylopii*, decreased to 2,441, 1,027 and 540 Has, respectively, Siracusa and Catania remain the provinces where biological fight is mainly performed, followed by an increasing number of farms in Agrigento province. Hence it is indirectly confirmed that the location of the Biofactory (Ramacca, Catania) is in line with the geographical distribution of its real users.

The profile of the more than 298 farms that, in 2012, took advantage of the service of the Biofactory of Ramacca is best represented in figure 11. Companies that follow programs of integrated biological defense or integrated fight in citrus and benefit of the insects provied by the biofactory have predominantly a size less than 5 hectares (161, 48 and 27 farms can be listed for *A. melinus*, *L. dactylopii* and *C. montrouzieri*, respectively). Medium-sized companies were those that, in 2012, performed more seasonal launches of *Aphytis melinus* with an average of about 3.8; but also the other companies showed average values (3–4 seasonal launches).

For *C. montrouzieri* the number of launches is inversely proportional to the company size, ranging from about 1 for small farms to 0.6 for larger ones.

A similar pattern was confirmed for *Leptomastix dactylopii* with about. 0.8 launches for companies under 5 Ha and. 0.4 launches for larger ones. Average launches < 1 reveal a partial use of insects (for organic control) that, in these cases, are not employed on the entire surface of the citrus grove. The new payment system had an impact also on the number of launches that, with reference to 2006–2011 data, appear in decline. This could be due to a more parsimonious use of the “organic product” but also to a kind of “users’ loyalty” (i.e., farmers despite the new regulation, continue to show a certain degree of satisfaction).

EVOLUTION OF SERVICE AND PROSPECTS FOR SEVEN YEARS FROM 2013 TO 2020.

The last items briefly discussed in the previous paragraph, led E.S.A. to review the current huge production and proceed, after an initial experimental phase, to the diversification of production, to improve the bouquet offered. In this contest E.S.A. has already started a project that will be developed in the period 2013–2020. In particular, the service aimed at breeding and producting huge quantities of *Aphytis melinus*, *Criptolaemus montrouzerii* and *Leptomastix dactylopii* is confirmed, re-thinking of new production levels, based on all the variables mentioned before. Moreover, seven additional experimental activities have also been designed one for each year, to be held simultaneously with the aforementioned base production, aimed at increasing the entomological list to be employed in other contests, as viticultural, ornamental and floricoltural. Each experiment involves the development of procedures for the breeding of the following auxiliaries (see below) to be performed, in proper conditions, for the production of huge quantities of insects.

1) *Cryptolaemus montruozierii* larvae (predators of *P. citri*, citrus mealybug);

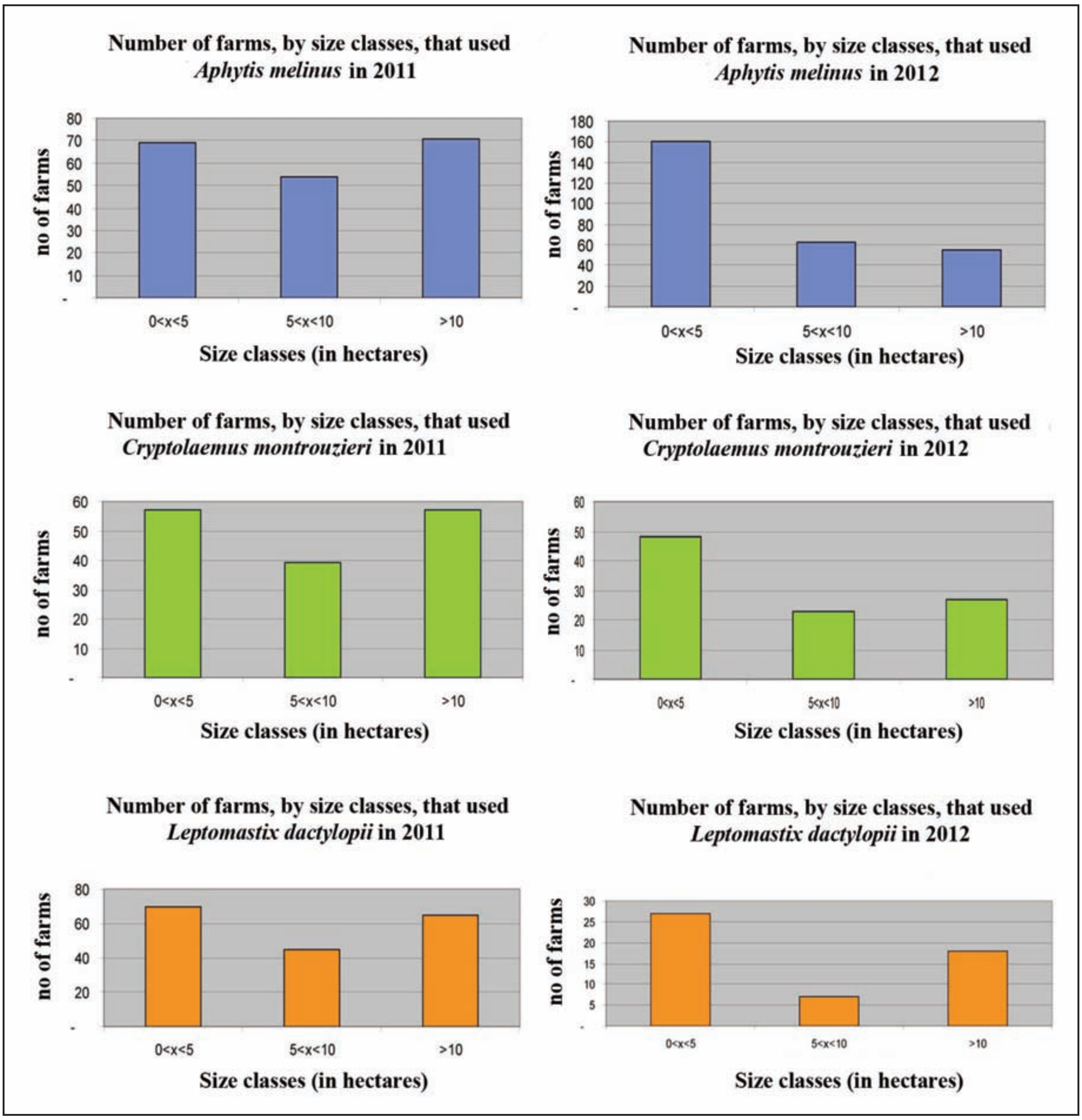

Figure 11. Number of companies, sorted by size classes, that used *Aphytis melinus*, *Criptolaemus montrouzerii* and *Leptomastix dactylopii* in 2011 and 2012 (Source E.S.A.).

2) *Chrysoperla carnea* Stephens, 1836 (Neuroptera Chrysopidae) predator of aphids (Benuzzi & Nicoli, 1988; Osservatorio agroambientale di Cesena, 1991; Nicoli & Galazzi, 2000);

3) *Anagyrus pseudococci* (Girault 1915) (Hymenoptera Encyrtidae) parasitoid of *Planococcus vitis* and ornamental mealybugs, *P. ficus*, *Pseudococcus longispinus*, *Ps. affinis*, *Rhizoecus falcifer* (Avidov et al., 1967; Rosen & Rössler, 1966; Islam & Jahan, 1993a, b; Blumberg et al., 1995; Islam & Copland, 1997, 2000).

4) *Encarsia formosa* Gahan, 1924 (Hymenoptera Aphelinidae), parasitoid of whiteflies as *Trialeurodes vaporarorium* (Westwood, 1856) (Hemiptera Aleyrodidae);

5) *Lindorus lophantae* (Blaisdell, 1892) (Coleoptera Coccinellidae) (generic predator of mealybugs, also active against *Aonidiella aurantii*);

6) *Orius laevigatus* (Fieber, 1860) (Hemiptera Anthocoridae) predator of thrips (Tawfik & Ata, 1973; Tavella et al., 1991; Villevieille & Millot, 1991; Chatnbers et al., 1993; Vacante & Tropea Garzia, 1993a-b; Meiracker van den, 1994; Alauzet et al., 1994; Tavella et al., 1994; Frescata & Mexia, 1995; Tommasini & Nicoli, 1995);

7) larvae of *Chilocorus bipustulatus* (Linnaeus, 1758) (Coleoptera Coccinellidae)(predators of *Coccus esperidum* (brown soft scale), *Ceroplastes sinenesis* (Chinese wax scale), *Ceroplastes rusci* (fig wax scale), *Saissetia oleae* (Black scale), *Carnuaspis bekii* (Purple scale), *Aspidiotus blacks* (Oleander scale), *Chrisomphalus dictyospermi* (Morgan's scale), *Aonidiella aurantii* (California red scale).

REFERENCES

Abdelrahman I., 1974. Growth, development and innate capacity for increase in *Aphytis chrysomphali* Mercet and *A. melinus* DeBach, parasites of California red scale, *Aonidiella aurantii* (Mask.) in relation to temperature. Australian Journal of Zoology, 22: 213–230.

Alauzet C., Dargagnon D. & Malausa J.C., 1994. Bionomics of the polyphagous predator: *Orius laevigatus* (Het.: Anthocoridae). Entomophaga, 39: 33–40.

Avidov Z., Rössler Y. & Rosen D., 1967. Studies on an Israel strain of, *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae). II. Some biological aspects, Entomophaga, 12: 111–118.

Blumberg D., Klein M. & Mendel Z., 1995. Response by encapsulation of four mealybug species (Homoptera: Pseudococcidae) in parasitization by, *Anagyrus pseudococci*. Phytoparasitica, 23: 157–163.

Celli G., Maini S. & Nicoli G., 1991. La fabbrica degli insetti. Franco Muzzio Editore, Padova, 333 pp.

Chandler L.D., Meyerdirk D.E., Hart W.G. & Garcia R.G., 1980. Laboratory studies on the development of the parasite, *Anagyrus pseudococci* (Girault) on insectary-reared, *Planococcus citri* (Risso), Southwest Entomology, 5: 99–103

Chatnbers R.J., Long S. & Helyer N.L., 1993. Effectiveness of *Orius laevigatus* (Hem.: Anthocoridac) for the control of *Frankliniella occidentalis* on cucumber and pepper in the UK. Biocontrol Science and Technology, 3: 295–307.

Chiri A.A., 1987. Enemigos naturales de los Afidos: Depredadores. Manejo Integrado de Plagas. En: Revista del Proyecto MIP/CATIE No. 4 pág 32. 1987.

Collier T.R., 1995. Host feeding, egg maturation, resorption, and longevity in the parasitoid *Aphytis melinus* (Hymenoptera: Aphelinidae). Annals of the Entomological Society of America, 88: 206–214.

De Bach P. & Argyriou L.C., 1967. The colonization and success in Greece of some imported *Aphytis* spp. (Hym.: Aphelinidae) parasitic on citrus scale insects (Hom. Diaspididae). Entomophaga, 12: 325–342.

De Bach P., Rosen D. & Kennet C.E., 1969. Biological control of coccids by introduced natural enemies. Huffaker C.B. (Eds.). Biological Control, 165–194.

Difesa integrata Agrumi - Regione Siciliana - Assessorato Agricoltura e Foreste-Osservatorio per le malattie delle piante, Acireale.

Flanders S.E., 1953. Aphelinid biologies with implications for taxonomy. Annals of the Entomological Society of America, 46: 84–94.

Flint M.L., 1991. Integrated Pest Management for *Citrus*. University of California, Division of Agriculture and Natural Resources, No. 3303, 144 pp.

Frescata C. & Mexia A., 1995. Biological control of Westem Flower Thrips with *Orius laevigatus* (Heteroptera Anthocoridae) in organic strawberries in Portugal. In: Parker B.L. et al. (Eds.) - Thrips biology and management. Plenum Press, New York, 249 pp.

Goidànich G., Bruno Casarini B. & Ugolini A., 1990. La difesa delle piante da frutto. Bologna, Edizioni agricole, 1202 pp.

Gottlieb Y., Zchorl-Feln E., Faktor O. & Rosen D., 1998. Phylogenetic analysis if parthenogenetic-inducing Wolbachia in the genus *Aphytis* (Hymenoptera: Aphelinidae). Insect Molecular Biology, 7: 393–396.

Grafton-Cardwell E.E. & Reagan C.A., 1995. Selective use of insecticides for control of armored scale (Homoptera: Diaspididae) in San Joaquin Valley California Citrus. Journal of Economic Entomology, 88: 1717–1725.

Greco G., 2014a. A biofactory in Sicily for the biological eradication of the *Ceratitis capitata* in the Mediterranean countries. www.entesviluppoagricolo.it

Greco G., 2014b. La Biofabbrica di Ramacca. www.entesviluppoagricolo.it

Hare J.D. & Luck, R.F., 1994. Environmental variation in physical and chemical cues used by the parasitic wasp, *Aphytis melinus*, for host recognition. Entomologia Experimentalis et Applicata, 72: 97–108.

Heimpel G.E. & Rosenheim J.A., 1995. Dynamic host feeding by the parasitoid *Aphytis melinus*: the balance between current and future reproduction. Journal of Animal Ecology, 64: 153–167.

Hoffmann M.P. & Frodshan A.C., 1993. Natural Enemies of Vegetable Insect Pest, Cooperative Extensión, Cornell University, Ithaca, N.Y., 63 pp.

Hodek I. & Honek A., 1996. Ecology of Coccinellidae, Dordrecht, Kluwer Academic Publishers.

Islam K.S. & Copland M.J.W., 1997. Host preference and progeny sex ratio in a solitary koinobiont mealybug endoparasitoid, *Anagyrus pseudococci* (Girault), in response to its host stage. Biocontrol Science and Technology, 7: 449–456.

Islam K.S. & Copland M.J.W., 2000. Influence of egg load and oviposition time interval on the host discrimination and offspring survival of, *Anagyrus pseudococci* (Hymenoptera: Encyrtidae), a solitary endoparasitoid of citrus mealybug, *Planococcus citri* (Hemiptera: Pseudococcidae). Bulletin of Entomological Research, 90: 69–75.

Islam K.S. & Jahan M., 1993a. Oviposition and development of the mealybug parasitoid, *Anagyrus pseudococci* (Girault) at different constant temperatures. Pakistan Journal of Scientific and Industrial Research, 36: 322–324.

Islam K.S. & Jahan M., 1993b. Influence of honeydew of citrus mealybug (*Planococcus citri*) on searching behavior of its parasitoid, *Anagyrus pseudococci*, Crop Protection, 63: 743–746.

Masutti S. & Zangheri S., 2001. Entomologia generale ed applicata. Padova, Cedam, 978 pp.

Luck R.F., Podoler H. & Kfir R., 1982. Host selection and egg allocation behavior by *Aphytis melinus* and *Aphytis lingnanensis*: comparison of two facultatively gregarious parasitoids. Ecological Entomology, 7: 397–408.

Luck R.F. & Podoler H., 1985. Competitive exclusion of *Aphytis lingnanensis* by *Aphytis melinus*: potential role of host size. Ecology, 66: 904–913.

Luck R.F., Forster L.D. & Morse J.G., 1996. An ecologically based IPM program for citrus in California's San Joaquin Valley using augmentative biological control. Proceedings International Society of Citriculture, pp. 499–503.

Ferrari M., Marcon E. & Menta A., 2000. Lotta biologica. Controllo biologico ed integrato nella pratica fitoiatrica. Edagricole, Bologna, 366 pp.

Ferrari M., Marcon E. & Menta A., 2006. Fitopatologia, entomologia agraria e biologia applicata. Bologna, Edagricole.

Mario Muccinelli, 2006. Prontuario dei fitofarmaci. Undicesima edizione. Bologna, Edagricole, 2006.

Massimo Benuzzi & Giorgio Nicoli (1988). *Chrysoperla carnea*. In: Lotta biologica e integrata nelle colture protette (Strategie e tecniche disponibili). Centrale Ortofrutticola alla Produzione, Cesena, 73–78.

Meiracker R.A.F. van den, 1994. Induction and terrnination of diapause in *Orius* predatory bugs. Entomologia experimentalis et applicata, 73: 127–137.

Milán Vargas O., 1999. *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae) como Control Biológico de la Chinche Harinosa Rosada del Hibiscus.

Moreno D.S. & Luck R.F., 1992. Augmentative releases of *Aphytis melinus* (Hymenoptera: Aphelinidae) to suppress California red scale (Homoptera: Diaspididae) in southern California lemon orchards. Journal of Economic Entomology, 85: 1112–1119.

Murdoch W.W., Briggs C.J. & Nisbet R.M., 1996. Competitive displacement and biological control in parasitoids: a model. American Naturalist, 148: 807–826.

Nicoli G. & Galazzi D., 2000. *Chrysoperla carnea*. In: Nicoli G. & Radeghieri P. (a cura di). Gli ausiliari nell'agricoltura sostenibile. Calderini Edagricole, Bologna.

Opp S.B. & Luck R.F., 1986. Effects of host size on selected fitness components of *Aphytis melinus* and *Aphytis lingnanensis* (Hymenoptera, Aphelinidae). Annals of the Entomological Society of America, 79: 700–704.

Orphanides G.M., 1984. Competitive displacement between *Aphytis* spp. (Hym. Aphelinidae) parasites of the California red scale in Cyprus. Entomophaga, 29: 275–281.

Osservatorio agroambientale di Cesena (1991). *Crisope*. In: Guida al riconoscimento degli organismi utili in agricoltura. Centro Servizi Avanzati per l'Agricoltura (Centrale Ortofrutticola di Cesena) e dell'Osservatorio agroambientale di Cesena, Bologna, 40–41.

Pasotti L., Perrotta G., Raciti E., Saraceno F., Sciacca V. & Tumminelli R., 2004. Validazione e applicazione in Sicilia Orientale di un modello di sviluppo della cocciniglia rossa forte degli agrumi, *Aonidiella aurantii* (Maskell), basato sull'accumulo di gradigiorno. Atti III Giornate di studio - Metodi numerici, statistici e informatici nella difesa delle colture agrarie e delle foreste. Firenze 24-26 nov. 2004: 177–181.

Pekas A., Tena A., Aguilar A. & Garcia-Marí, F., 2010. Effect of Mediterranean ants (Hymenoptera: Formicidae) on California red scale *Aonidiella aurantii* (Hemiptera: Diaspididae) populations in citrus orchards. Environmental Entomology, 39: 827–834.

Penny J. & Cranston P.S., 2006. Lineamenti di entomologia. Bologna, Zanichelli, 514 pp.

Pina T., Martínez B. & Verdú, M.J., 2003. Field parasitoids of *Aonidiella aurantii* (Homoptera: Diaspididae) in Valencia (Spain). IOBC/WPRS Bulletin, 26: 109–115.

Pina T., 2007. Control biológico del piojo rojo de California *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) y estrategias reproductivas de su principal enemigo natural *Aphytis chrysomphali* Mercet (Hymenoptera: Aphelinidae), 384 pp.

Pina T. & Verdú M.J., 2007. El piojo rojo de California *Aonidiella aurantii* (Maskell) y sus parasitoides en cítricos de la Comunidad Valenciana. Boletín de Sanidad Vegetal Plagas, 33: 357–368.

Pollini A., Ponti I. & Franco Laffi F., 1988. Fitofagi delle piante da frutto. Verona, Edizioni L'informatore agrario, 188 pp.

Pollini, 1998. Manuale di entomologia applicata. Bologna, Edagricole, 1462 pp.

Reeve J.D., 1987. Foraging behavior of *Aphytis melinus*: effects of patch density and host size. Ecology, 68: 530–538.

Rodrigo E. & García-Marí F., 1992. Ciclo biológico de los diaspinos de cítricos *Aonidiella aurantii* (Mask.), *Lepidosaphes beckii* (Newm.) y *Parlatoria pergandei* (Comst.) en 1990. Boletín de Sanidad Vegetal Plagas, 18: 31–44.

Rodrigo E. & García-Marí F., 1990. Comparación del ciclo biológico de los diaspinos *Parlatoria pergandii*, *Aonidiella aurantii* y *Lepidosaphes beckii* (Homoptera, Diaspididae) en cítricos. Boletín de Sanidad Vegetal Plagas, 16: 25–35

Rodrigo M.E., Garcia-Mari F., Rodriguez-Reina J.M. & Olmeda T., 2004. Colonization of growing fruit by the armored scales *Lepidosaphes beckii*, *Parlatoria pergandii* and *Aonidiella aurantii* (Hom., Diaspididae). Journal of Applied Entomology, 128: 569–575.

Rosen D. & Rössler Y., 1996. Studies on an Israel strain of , *Anagyrus pseudococci* (Girault) (Hymenoptera, Encyrtidae). I. Morphology of the adults and developmental stages. Entomophaga, 11: 269–277.

Rosen D., DeBach P. & Junk W. (Eds.), 1979. Species of *Aphytis* of the World (Hymenoptera: Aphelinidae), 801 pp.

Rosen D. & Eliraz A., 1978. Biological and systematic studies of developmental stages in *Aphytis* (Hymenoptera: Aphelinidae). I. Developmental history of *Aphytis chilensis* Howard. Hilgardia, 46: 77–95.

Sorribas J., Rodriguez R. & Garcia-Marí F., 2010. Parasitoid competitive displacement and coexistence: linking spatial and seasonal distribution with climatic conditions. Ecological Applications, 20: 1101–1113.

Sorribas J., Rodriguez R., Rodrigo E. & Garcia Marí F., 2008. Niveles de parasitismo y especies de parasitoides del piojo rojo de California *Aonidiella aurantii* (Hemiptera: Diaspididae) en cítricos de la Comunidad Valenciana. Boletín de Sanidad Vegetal Plagas, 34: 201–210.

Tavella L., Arzone A. & Alma A., 1991. Researches on *Orius laevigatus* (Fieb.), a predator of *Frankliniella occidentalis* (Perg.) in greenhouses. A preliminare note. Bulletin IOBC/WPRS, 14: 65–72.

Tavella L., Arzone A. & Giordano V., 1994. Indagini biologiche su *Orius laevigatus* (Fieber) (Rhynchota: Anthocoridae) predatore di *Frankliniella occidentalis* (Pergande) (Thysanoptera). Atti XVII Congresso Nazionale Italiano di Entomologia Udine 13–18 giugno 1994: 519–521.

Tawfik M.ES. & Ata A.M., 1973. The life-history of *Orius laevigatus* (Fieber). Bulletin de la Société entomologique d'Égypte, 57: 145–151.

Tena A. & Garcia-Mari F., 2011. Current situation of citrus pests and diseases in the Mediterranean Basin. IOBC/WPRS Bulletin, 62: 365 –378.

Tingle C.C.D. & Copland M.J.W., 1988. Predicting development of the mealybug parasitoids, *Anagyrus pseudococci*, *Leptomastix dactylopii* and *Leptomastidea abnormis* (Hym. Encyrtidae) under glasshouse conditions. Entomologia Experimentalis et Applicata, 46, 19–28.

Tingle C.C.D. & Copland M.J.W., 1989. Progeny production and adult longevity of the mealybug parasitoids , *Anagyrus pseudococci*, *Leptomastix dactylopii* and, *Leptomastidea abnormis* (Hym. Encyrtidae) in relation to temperature. Entomophaga 34: 111–120.

Tommasini M.G. & Nicoli G., 1995. Evaluation of *Orius* spp. as biological control agents of thrips pests: Initial experiments on the existence of diapause in *Orius laevigatus*. Medicine Faculty Landbouw, University Gent (B), 60/3a: 901–908.

Tremblay E., 1988. Entomologia applicata, vol. II, pars I. 2a ed. Napoli, Liguori Editore, 1988.

Tumminelli R., Conti F., Saraceno F., Raciti E. & Schilirò, R., 1996. Seasonal development of California red scale (Homoptera: Diaspididae) and *Aphytis melinus* DeBach (Hymenoptera: Aphelinide) on citrus in Eastern Sicily. Proceedings of the International Society of Citriculture, Sun City, South Africa, 493–498.

Vacante V. & Tropea Garzia G., 1993a. Prime osservazioni sulla risposta funzionale di *Orius laevigatus* (Fieber) nel controllo di *Frankliniella occidentalis* (Pergande) su peperone in serra fredda. Colture protette, 22 (suppl. n.1): 33–36.

Vacante V. & Tropea Garzia G., 1993b. Ricerche di laboratorio sulla biologia di *Orius laevigatus* (Fieber). Colture protette, 22 (suppl. n.1): 37–38.

Vacas S., Alfaro C., Navarro-Llopis V. & Primo J., 2009. The first account of the mating disruption technique for the control of California red scale, *Aonidiella aurantii* Maskell (Homoptera: Diaspididae) using new biodegradable dispensers. Bulletin of Entomological Research, 99: 415–423.

Vanaclocha P., Urbaneja A. & Verdú M.J., 2009. Mortalidad natural del piojo rojo de California, *Aonidiella aurantii*, en cítricos de la Comunidad Valenciana y sus parasitoides asociados. Boletín de Sanidad Vegetal Plagas, 35: 59–71.

Viggiani G., 1977. Lotta biologica e integrata nella difesa fitosanitaria vol. I e vol. II. Liguori Editore, Napoli, 517+445 pp.

Villevieille M. & Millot P., 1991. Lotte biologique contre *Frankliniella occidentalis* avec *Orius laevigatus* sur fraisier. Bulletin IOBC-WPRS, 14: 57–64.

Walker A. K., 1994. A review of the pest status and natural enemies of *Thrips palmi*. Biocontrol News and Information, 15: 7N-1ON.

Vacante V. & Benuzzi M., 2004. Produzione massale e impiego dei principali ausiliari nella lotta biologica in orticoltura e agrumicoltura. Aracne Editrice, Roma, 33 pp.

Walde S.J., Luck R.F., Yu D.S. & Murdoch W.W., 1989. A refugee for red scale: the role of size-selectivity by a parasitoid wasp. Ecology, 70: 1700–1706.

Yu D.S., 1986. The interactions between California red scale *Aonidiella aurantii* (Maskell), and its parasitoids in citrus groves of inland southern California. Ph.D. dissertation, University of California, Riverside, 105 pp.

Yu S. & Luck R.F., 1988. Temperature-dependent size and development of California red scale (Homoptera: Diaspididae) and its effect on host availability for the ectoparasitoid, *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae). Environmental Entomology, 17: 154–161.