Bulletin of Insectology **69** (1): 13-20, 2016
ISSN 1721-8861

A Mediterranean silvo-pastoral system supporting beehive health and productivity

Ignazio Floris[1], Simonetta Bagella[2], Maria Carmela Caria[2], Luca Ruiu[1], Franco Buffa[1], Alberto Satta[1]
[1]*Dipartimento di Agraria, sezione di Patologia vegetale ed Entomologia, Università di Sassari, Italy*
[2]*Dipartimento di Scienze della Natura e del Territorio, Università di Sassari, Italy*

Abstract

The biological, productive and sanitary conditions were investigated in an experimental apiary in order to evaluate the aptitude of the Mediterranean silvo-pastoral systems to the maintenance and exploitation of bee colonies through the best hive management practices. Observations on bee colony dynamics, honey production in storing supers, *Paenibacillus larvae* (White 1906) spores detection and *Varroa destructor* Anderson et Trueman infestation were conducted. The annual trend of bee population dynamics showed two typical peaks (spring and autumn). The total average honey production was 70 kg per hive. From a sanitary point of view, colonies looked normal and healthy with the sole exception of *V. destructor* infestations that were regularly kept under the intervention threshold by hive management operations. The results suggest that the Mediterranean agro-silvo-pastoral systems may be particularly suitable for productive and healthy apiculture.

Key words: honey production, honeybees, plant communities, pollen spectra, silvo-pastoral systems.

Introduction

In addition to their high value for worldwide honey and other beehive products, honey bees play a major role in plant biodiversity conservation in natural and semi-natural ecosystems and in modern agriculture productivity through their essential pollination services (vanEngelsdorp *et al*., 2010).

The growing concern for the enigmatic honey bee colony decline, experienced both in the United States and in Europe, is triggering extensive studies to identify the possible abiotic and biotic causal agents, including pathogens and parasites. It is well known that pest management in agriculture frequently causes negative side effects on honey bees (Desneux *et al*., 2007; Maini *et al*., 2010). On the other side, bees are affected by the action of parasitic mites (*Varroa destructor* Anderson et Trueman, *Acarapis woodi* Rennie, *Tropilaelaps* spp.), scavengers (especially beetles) and various microbial pathogens including bacteria [*Paenibacillus larvae* (White 1906), *Melissococcus plutonius* (ex White) Bailey et Collins corrig. Truper et de Clari], fungi [*Nosema* spp., *Ascosphaera apis* (Maasen ex Claussen) Olive et Spiltoir] and viruses (deformed wing virus, acute paralysis virus, black queen cell virus, sacbrood virus and others) (Higes *et al*., 2006; Nazzi *et al*., 2012; Gaggìa *et al*., 2015). Pesticides, particularly insecticides, may affect the honeybee immune system and increase their susceptibility to pathogens (Alaux *et al*., 2010a; Di Prisco *et al*., 2013; Doublet *et al*., 2015). Moreover, the increasing use of fertilizers and pesticides in agriculture, the progress in destruction and fragmentation of natural and semi-natural habitats and the intensification of land use reduce the availability of suitable nesting sites, adequate forage and nourishment, thereby significantly affecting the fitness of honeybees and other pollinators (Rathcke and Jules, 1993; Quaranta *et al*., 2004; Tscharntke *et al*., 2005; Kremen *et al*., 2007). These negative factors can be mitigated by implementing agro-ecosystem management with integrated or organic farming, ensuring natural and/or semi-natural habitat conservation and improving farmland biodiversity (Steffan-Dewenter and Westphal, 2008; Decourtye *et al*., 2010). These conditions can be achieved in semi-natural ecosystems such as the Mediterranean agro-silvo-pastoral systems that provide different services to human societies (Millennium Ecosystem Assessment, 2005). This typical Mediterranean system integrates forests, tree plantations and herbaceous crops with grazing livestock, thus ensuring a significant degree of diversification and combination of productive factors (Caballero *et al*., 2009).

The typical environment of Mediterranean agro-silvo-pastoral systems is marked by two fundamental features: the Mediterranean character of the climate (dry summers and somewhat cold winters) and the low fertility of the soil, making intensive farming unsustainable and unprofitable. In these systems, the most efficient strategy is diversification and the use of every natural resource with a minimum input of energy and materials (Olea and San Miguel-Ayanz, 2006). Moreover these systems can comply with societal needs such as landscape and biodiversity conservation, or with ethical concerns on food production. Hence, these systems can easily adapt to market changes (Bernués *et al*., 2011) being particularly prone to a multiple use of the resources (Gómez Sal and González García, 2007). In this context, honey production represents a traditional resource in harmony with agro-pastoral activities and ecosystem conservation (Croitoru and Merlo, 2005).

In the present work, the biological (colony development dynamics), productive (honey production and major pollen sources identification) and sanitary conditions (particularly American foulbrood and Varroosis), were investigated within a group of beehives in order to evaluate the aptitude of the Mediterranean silvo-pastoral systems in the maintenance and exploitation of bee colonies through the best hive management practices. The value of different vegetation types for honey production in the same site and period were reported earlier (Bagella *et al*., 2013b).

Materials and methods

Study area

The study area was located in a silvo-pastoral landscape on gently sloping land in the municipalities of Monti (Gallura, NE Sardinia, Italy). The area belongs to the Meso-Mediterranean phytoclimatic belt, and overlies an Upper Carboniferous-Permian granitic substrate at an elevation of 250-300 m a.s.l., where annual rainfall is 632 mm and mean temperature is 14.2 °C. The soil is predominantly Typic Dystroxerept (Bagella *et al.*, 2014).

The experimental apiary was located in the centre of a circular area (1.5 km radius, 40°48'55"N 9°14'55"E) whose size was established on previous knowledge on the honey bees activity range, known to be concentrated within 1-2 km from the apiary in complex landscapes with annual and perennial habitat types (Villanueva-Gutiérrez, 2002; Steffan-Dewenter and Kuhn, 2003).

The potential vegetation of the study site is mainly represented by cork oak woods (*Violo dehnhardtii-Quercetum suberis*) (Bagella and Caria, 2011). Nevertheless, the area is mainly occupied by replacement communities and crops. The best represented vegetation types (40% cover) are open grasslands, dominated by grasses, legumes (e.g. *Trifolium nigrescens* Viviani), and forbs (e.g. *Echium plantagineum* L.) that develop in weed fallow, frequently cultivated with annual forage crops to make hay. Extensive wooded grasslands are mainly characterized by *Trifolium subterraneum* L., garrigues are dominated by *Cistus monspeliensis* L. and *Lavandula stoechas* L., shrub communities by *Cytisus villosus* Pourret, tall maquis by *Arbutus unedo* L. and *Erica arborea* L.. The landscape is also characterized by the presence of natural hedges with *Rubus ulmifolius* Schott, *Rhamnus alaternus* L., *Prunus spinosa* L. and *Crataegus monogyna* Jacquin. Monospecific planted *Eucalyptus* sp. hedges are also present (Bagella *et al.*, 2013b). Detailed vegetation and flowering features of this area are given in Bagella *et al*. (2013b).

Hives management and data collection

The experimental apiary consisted of 5 hives, prepared with queens of *Apis mellifera ligustica* breed and with an homogeneous genetic profile (sisters), provided by a local specialist breeder.

The experiments started in July 2009 and ended in July 2010. Daily rainfall and temperatures were recorded during the whole experimental period by a weather station localized 3 km far from the apiary (40°47'12"N 9°13'20"E), thus providing data on the potential honeybee flying days as a result of the weather effects on honeybees foraging activities and the limitations due to adverse climatic factors (Burrill and Dietz, 1981).

Main hive management operations during the experimental period involved supplemental feeding of honey bee colonies, swarming control, monitoring of the main honey bee pests, pathogens and parasites, and targeted treatments against *V. destructor*. Supplemental feeding included a weekly administration of 50% sugar syrup (500 ml) from 14th August to 26th September in each hive by employing a bag feeder (7 consecutive administrations) during summer 2009. In 2010, supplemental feeding was limited to the three weaker colonies to which 50% sugar syrup (500 ml) were administered weekly in each hive from 22nd January to 4th February (3 consecutive administrations).

Swarming control was based on the weekly removal of royal cells during spring from 19th March to 15th July. Sporadically, some honey or brood comb frames were removed from the beehives more exposed to imminent swarming risks.

According to the integrated pest management criteria, *Varroa* mite control was based on the monitoring and evaluation of *Varroa* infestation level through the crossed sampling of sealed worker brood (Floris, 1991), prefixing a critical infestation threshold of 8-10% for treatment timing. For this purpose, mite infestation levels of worker sealed brood were surveyed every month. Once the mean infestation level of the apiary reached the threshold, one of the following acaricides was applied: Apiguard® (a.i. thymol) in summer and Apivar® (a.i. amitraz) in winter.

The colony development dynamics was monitored every 2 weeks through the estimation of the sealed brood extension and the amount of adult bees. For this purpose, one-sixth of a Dadant-Blatt frame (188 cm^2) was used as a unit of measure converted in the graph of the results section in number of sealed cells and adult bees obtained by multiplying the number of sixth of each matrix for 780 and 254, respectively (Marchetti, 1985).

The pollen sources visited by the honeybees during the two main honey flows (autumn season: October-November, and spring season: March-June) were determined through the identification of pollen types gathered from the hind leg pollen loads of about 20-25 foragers (4-5 per hive) collected at the entrance of the hives on each sampling date. Pollen analyses were carried out following the method of the International Commission of Bee Botany (Louveax *et al*., 1978; Von der Ohe *et al*., 2004). Pollens were identified by comparing the samples with reference slides from the collection of the *Dipartimento di Agraria* (University of Sassari) and with the Atlas of Mediterranean Melissopalinology (Ricciardelli D'Albore, 1998). Since identification of pollen is not always possible at the species level, terms 'pollen type', 'group' or 'form' are used instead. These terms refer to the types of pollen characteristic of a particular species (e.g. *Trifolium repens*) whose pollen grains are similar to other species of the same genus or to a group of similar genera (Persano Oddo and Ricciardelli D'Albore, 1989). Forager samples were collected in the following dates: 24th October, 13th and 20th November, 30th December in the autumn season, 19th and 26th March, 13th, 14th, 26th and 30th April, 13th, 21st and 28th May, 25th June in the spring season. A comparison between the pollen spectra of honey and plant species visited by bees as nectar resources during the monitoring period is reported elsewhere (Bagella *et al*., 2013b).

Concerning the sanitary status of the colonies, in addition to the monitoring of sealed brood *Varroa* infestation level, any symptom of brood and adult bees referable to the main hive diseases caused by bacteria, fungi and viruses, were recorded during bee population dynamic surveys by visual diagnosis of brood combs. Moreover, monitoring surveys for the detection of the American foulbrood were conducted twice during the trial (November 2009 and April 2010) including both the symptom observations on sealed brood and the detection of *P. larvae* spores on approximately 100 adult bees collected from combs with unsealed brood frames from each hive (Hornitzky and Karlovskis, 1989; Gende *et al*., 2011).

Quantitative honey production stored in supers was monitored during the experiments for each hive. The difference between the weight of the full honey super at removal and its weight before being placed onto the hive was used to calculate honey production.

Results

In the study period, maximum and minimum average daily temperatures exceeded the 12 °C threshold, representing the minimal thermal level for bee foraging activity, respectively for 334 and 249 days. In 2010, January was the coldest month (T mean: 6.8 °C, T min: 3.8 °C, T max: 9.8 °C), July was the warmest (T mean: 25.4 °C, T min: 18.1 °C, T max: 33.7 °C). The total precipitation was 1032 mm, distributed in 102 days. September was the rainiest month (197 mm) followed by January (119 mm) and November (98 mm). Late in autumn, bees continued their pollen and nectar foraging activities until the middle of December when maximum average temperature reached 12.8 °C. At the end of winter, foraging flights were recorded from the middle of February when the mean of maximum temperature was 14.9 °C.

The trend of bee population dynamics respectively for brood and adults during the whole annual cycle (July 2009 - July 2010) is shown in figure 1. Two peaks, in spring (April/May) and autumn (October), were observed. Remarkably, the brood was present throughout the entire year, although with significant seasonal variations, particularly in winter (December-February) and in correspondence to unfavourable climatic conditions during the spring (i.e. the first week of April).

The total honey production reached about 69.1 ± 23.7 kg on average per hive (± SD), mainly obtained during spring (62.5 kg suite honey) than in autumn (6.6 kg strawberry tree, *A. unedo* L., honey, renowned as bitter honey) (table 1). The spring production lasted from the middle of March to the middle of June with the extraction of 5.2 ± 0.6 distinct supers on average per hive (from a minimum of 3 to a maximum of 6).

Table 1. Honey seasonal production (kg) (SD = standard deviation).

Hive	Autumn	Spring
1	11.3	70.0
2	7.0	63.8
3	3.1	73.3
4	1.5	26.8
5	10.2	78.6
Mean	6.6	62.5
SD	4.3	20.7

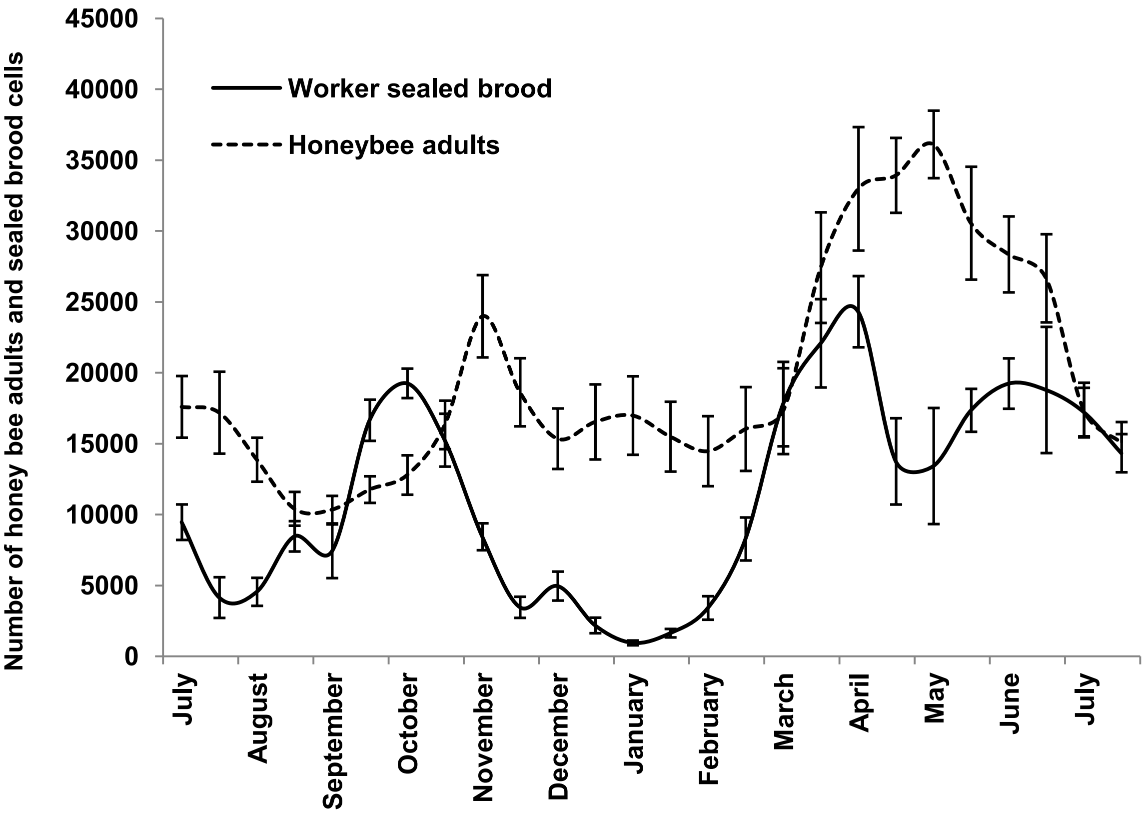

Figure 1. Number of honeybee adults (mean ± SE) and of worker sealed brood cells (mean ± SE) during the experiment (July 2009-July 2010).

The number of pollen sources exploited by the foragers was higher in spring than in autumn (30 *vs.* 8). In the latter season, the most frequent pollen loads were from *Hedera helix* L. in October, *Quercus suber* L. in November and *Cichorium intybus* L. in December (table 2) in spring, *R. alaternus* L, *Salix* sp. and *Erodium cicutarum* (L.) L'Hérit in March, *Trifolium repens* gr. and *C. monogyna* in April, *T. repens* gr., *C. monspeliensis* and *L. stoechas* in May, and finally *R. ulmifolius*, *Myrtus communis* L. and *C. intybus* in June (table 3).

During the trial, the mean mite infestation level of worker sealed brood reached three times the critical threshold (figure 2). In the first two cases, the peaks of infestation coincided with a minimum amount of sealed brood. The third peak was reached at the end of the trial when the mean amount of brood per hive was still quite high (no. of sealed brood cells 14320 ± 1346). Both treatments were followed by a significant reduction in the percentage of infestation level of sealed brood which were 0.58 ± 0.3 and 0.13 ± 0.1 at the end of the first

Table 2. Percent of pollen loads collected by honey bee adults in autumn (2009).

Pollen type	Month and number of bees examined		
	October	November	December
	21	49	6
Calendula arvensis	-	2.0	-
Cichorium intybus	19.0	14.3	83.3
Hedera helix	57.1	-	-
Quercus suber	-	79.6	-
Stachys 'form'	14.3	-	-
Smilax aspera	9.5	-	-
Composite 'pollen type'	-	4.1	-
Ranunculus sardous	-	-	16.7

Table 3. Percent of pollen loads collected by honey bee adults in spring (2010).

Pollen type	Month and number of bees examined			
	March	April	May	June
	50	99	70	27
Brassica f. <20-25>	4.0	-	-	-
Capsella bursa-pastoris	2.0	-	-	-
Carduus 'form'	-	-	2.9	-
Cercis siliquastrum	-	-	1.4	-
Cichorium intybus	-	-	-	14.8
Cistus incanus	-	-	2.9	-
Cistus monspeliensis	-	-	22.8	-
Crataegus monogyna	4.0	29.3	-	-
Daucus carota	-	-	-	7.4
Echium sp.	-	-	-	3.7
Erica arborea	2.0	-	-	-
Erodium cicutarium	16.0	-	-	-
Eucalyptus sp.	-	-	1.4	3.7
Euphorbia helioscopia	2.0	-	-	-
Fraxinus sp.	-	2.0	-	-
Galactites tomentosa	-	-	-	7.4
Hieracium 'form'	4.0	-	-	11.1
Lavandula stoechas	-	4.0	17.1	-
Myrtus communis	-	-	-	22.2
Prunus spinosa	12.0	-	-	-
Pyrus amygdaliformis	-	1.0	-	-
Rhamnus alaternus	30.0	-	-	-
Rubus ulmifolius	-	-	-	25.9
Ruscus aculeatus	4.0	-	-	-
Salix sp.	18.0	-	-	-
Smyrnium rotundofolium	-	1.0	-	-
Teline monspessulana	-	6.1	-	-
Trifolium repens 'group'	-	44.4	51.4	3.7
Trifolium incarnatum	-	12.1	-	-
Viburnum tinus	2.0	-	-	-

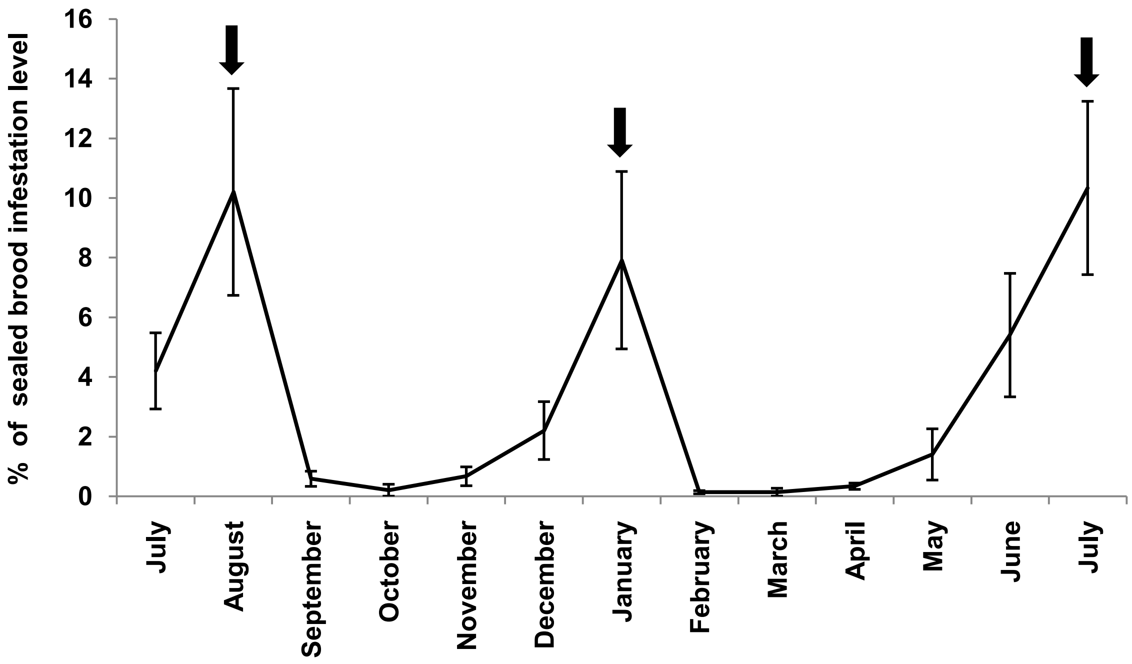

Figure 2. Trends (mean percentage ± SE) in mite infestation level of sealed brood during the experiment (July 2009-July 2010). Arrows show the treatment periods.

(Apiguard®) and the second (Apivar®) treatment, respectively. No apparent symptoms of bacterial and viral infections in the brood combs of colonies were noted during the trial. However, spores of *P. larvae* were detected in adult bees both in November 2009 and April 2010 although at low concentrations (27.7 ± 11.7 vs. 0.7 ± 0.3, respectively).

Discussion

The Mediterranean agro-silvo-pastoral systems are characterized by large habitat heterogeneity and the integration of different productive factors (San Miguel-Ayanz, 2005; Olea and San Miguel-Ayanz, 2006). Thanks to these features, a higher level of biodiversity can be detected in these systems, especially for pollinator species richness and abundance, in comparison with intensive agro-ecosystems (Benton *et al.*, 2003; Kleijn *et al.*, 2006; Potts *et al.*, 2006; Albrecht *et al.*, 2007; Bagella *et al.*, 2013a).

As a result of our study, the Mediterranean agro-silvo-pastoral systems appear to be well fitted for an adequate and healthy bee colony development during most of the year, resulting in abundant production of good quality honey. A "Mediterranean-type brood rhythm" in the colony development trend through seasons was observed (Ruttner, 1986). As expected, a summer slow-down was associated to drought and to lower flowering intensity, so that in this period colonies were fed weekly with 500 ml of 50% sugar syrup. The rapid increase in the queen oviposition rate, detected from the end of September, led colony population to reach adequate levels for the exploitation of *A. unedo* (strawberry fruit) flowering. This species is particularly abundant in the tall maquis referred to the association *Erico arboreae-Arbutetum unedonis*, where it reaches a cover of 75-100% (Biondi and Bagella, 2005). The production of strawberry tree honey, renowned as bitter honey, was favoured by low rainfall and mild temperatures in the period October-December. This honey represents a typical Mediterranean product with a traditional therapeutic use related to its potential bioactive properties (Floris *et al.*, 2007; Rosa *et al.*, 2011), which explains its higher market price (40-80 euro per kg).

Among species foraged by bees for pollen in autumn, *H. helix* was mainly present in *Cytisus* shrubs (*Telino monspessulanae-Cytisetum villosi* association), *C. intybus* in open grasslands (*Echio plantaginei-Galactition tomentosae*), *Quercus suber* in cork oak woods (*Violo dehnhardtii-Quercetum suberis* association) (Bagella *et al.*, 2013b). The unusual autumnal flowering of cork oak, mirrored by the presence of its pollen in pollen loads as already reported in literature (Camarda and Valsecchi, 2008), was probably induced as a side effect of the strong infestation of the lepidopteron defoliator *Lymantria dispar* (L.) occurring the previous year. Following the decline of strawberry tree nectar flow and temperature decrease, bee colonies entered the overwintering period which lasted exclusively between December and January. At the end of winter, the queen oviposition rate raised rapidly, taking advantage of early flowering of plant species rich in pollen and nectar (i.e. *E. arborea* and *Pyrus communis* L.) and of temperature increase. In this period, the stimulant feeding with sugar syrup was administered only for two weeks from the end of January to the three weaker families. As a result of abundant flowers in spring, honey deposition in honey combs started from the middle of March, so that the first honey extraction took place after one month (around the middle of April). At that time, the most visited plant species were *Salix purpurea* L., *R. alaternus* and several *Trifolium* spp. among which the most represented being *Trifolium michelianum* Savi and *T. nigrescens*, typical of semi-natural grasslands (Bagella *et al*., 2013a). All these species were visited by bees for foraging both pollen and nectar. From the beginning of April to early May, in addition to *Trifolium* spp., a highly relevant nectar source was *L. stoechas*. In fact, the pollen spectrum of honey collected in this period was consistent with unifloral lavender honey (Persano Oddo *et al*., 2000; Bagella *et al*., 2013b). In the same period, besides *Trifolium* spp., the major pollen source was *C. monogyna*, typical of the hedges. Honey produced in May and June was multifloral and derived from the preeminent nectar sources that were again represented by clovers and lavender species progressively replaced by *Echium italicum* L. and *E. plantagineum*. On the other side, major pollen sources for bees were *C. monspeliensis* in May and *M. communis* and *R. ulmifolius* in June.

During the study period, no significant bee health concerns raised, in contrast with usual expectations for agro-ecosystems where the use of pesticides can be intensive (Maini *et al*., 2010; VanEngelsdorp and Meixner, 2010). For the management of *Varroa* mites, usual procedures proven for the Mediterranean environment, especially in Sardinia, were employed (Floris *et al*., 2001; Floris *et al*., 2004; Satta *et al*., 2005) to keep mite infestation under the damage threshold (Floris, 1991). Anyway, the symptoms observed in colonies were never related to the parasitic mite syndrome (Shimanuki *et al*., 1994; Nazzi, *et al*., 2012).

Despite spores of the American foulbrood causal agent were detected in adult bees, no clinical symptoms of this pathogen were recorded in the brood. A recent study demonstrated the ubiquitous spread of *P. larvae* in Sardinia as it was detected in 95% of adult bees from different parts of the island (Gende *et al*., 2011). Remarkably, the spore concentration per adult bee was significantly lower than the threshold for the appearance of clinical symptoms of the disease, estimated to be around 3000 spores/adult bee (Gende *et al*., 2011). In addition, because the average value recorded in April 2010 was significantly lower than in November 2009, even if no treatments were performed, we can notice that bees were able to counteract infections by their innate defense mechanisms. It is known that the ability to counteract the American foulbrood depends mostly on the hygienic behaviour of bees as well as on their development speed that can reduce the spore load in the colony (Genersch, 2010). However, considering the importance of pollen quality and diversity in the immune response of honey bees (Alaux *et al*., 2010b) we cannot exclude that in the studied ecosystem the variety of resources and the high degree of floristic diversity may have contributed to maintaining honey bee health.

In relation to the technical management of beehives, the swarming control was the most costly operation. In fact, in spring (March-May) a weekly inspection of colonies for royal cells removal and brood or honey combs replacement was carried out.

Based on the occurring technical, biological and sanitary conditions, high honey production levels were achieved. This result is probably in relation also to the low density of apiaries in the studied area.

The main finding from the present study is that Mediterranean agro-silvo-pastoral systems are particularly suitable for apiculture in terms of resource availability throughout the year, which favours colony development, health status and productive fitness.

Acknowledgements

This study was financially supported by the Italian Ministry of Education, University and Research (MIUR): Research Project PRIN 2007, "Honeybee knowledge integration by developing and calibrating a simulation model for the hive".

References

ALAUX C., BRUNET J. L., DUSSAUBAT C., MONDET F., TCHAMITCHAN S., COUSIN M., BRILLARD J., BALDY A., BELZUNCES L. P., LE CONTE Y., 2010a.- Interactions between *Nosema* microspores and a neonicotinoid weaken honeybees (*Apis mellifera*).- *Environmental Microbiology*, 12: 774-782.

ALAUX C., DUCLOZ F., CRAUSER D., LE CONTE Y., 2010b.- Diet effects on honeybee immunocompetence.- *Biology Letters*, 6: 562-565.

ALBRECHT M., DUELLI P., MÜLLER C., KLEIJN D., SCHMID B., 2007.- The Swiss agri-environment scheme enhances pollinator diversity and plant reproductive success in nearby intensively managed farmland.- *Journal of Applied Ecology*, 44: 813-822.

BAGELLA S., CARIA M. C., 2011.- Vegetation series as a tool for the assessment of grassland ecosystem services in Mediterranean large-scale grazing systems.- *Fitosociologia*, 48: 47-54.

BAGELLA S., SALIS L., MARROSU G. M., ROSSETTI I., FANNI S., CARIA M. C., ROGGERO P. P., 2013a.- Effects of long-term management practices on grassland plant assemblages in Mediterranean cork oak silvo-pastoral systems.- *Plant Ecology*, 214: 621-631.

BAGELLA S., SATTA A., FLORIS I., CARIA M. C., ROSSETTI I., PODANI J., 2013b.- Influence of plant community composition and flowering phenology on honeybee foraging in a Mediterranean sylvo-pastoral ecosystem.- *Applied Vegetation Science*, 16: 688-697.

BAGELLA S., FILIGHEDDU R., CARIA M. C., GIRLANDA M., ROGGERO P. P., 2014.- Contrasting land uses in Mediterranean agro-silvo-pastoral systems generated patchy diversity patterns of vascular plants and below-ground microorganisms.- *Comptes rendus Biologies*, 337 (12): 717-724.

BENTON T. G., VICKERY J. A., WILSON J. D., 2003.- Farmland biodiversity: is habitat heterogeneity the key?- *Trends in Ecology and Evolution*, 18: 182-188.

BERNUÉS A., RUIZ R., OLAIZOLA A., VILLALBA D., CASASÙS I., 2011.- Sustainability of pasture-based livestock farming systems in the European Mediterranean context: Synergies and trade-offs.- *Livestock Science*, 139: 44-57.

BIONDI E., BAGELLA S., 2005.- Vegetazione e paesaggio vegetale dell'arcipelago di La Maddalena (Sardegna nord-orientale).- *Fitosociologia*, 42 (supplement 1): 3-99.

BURRIL R. M., DIETZ A., 1981.- The response of honeybees to variations in solar radiation and temperature.- *Apidologie*, 12: 319-328.

CABALLERO R., FERNÁNDEZ-GONZÁLES F., PÉREZ BADIA R., MOLLE G., ROGGERO P. P., BAGELLA S., D'OTTAVIO P., PAPANASTASIS V. A., FOTIADIS G., SIDIROPOULOU A., ISPIKOUDIS I., 2009.- Grazing systems and biodiversity in Mediterranean areas: Spain, Italy and Greece.- *Pastos*, 39: 9-154.

CAMARDA I., VALSECCHI F., 2008.- *Alberi e arbusti spontanei della Sardegna*.- Carlo Delfino, Sassari, Italy.

CROITORU L., MERLO M., 2005.- Mediterranean forest value, pp. 37-68. In: *Valuing Mediterranean forests: toward total economic value* (MERLO M., CROITORU L., Eds).- CABI Publishing, Wallingford, UK.

DECOURTYE A., MADER E., DESNEUX N., 2010.- Landscape enhancement of flora resources for honey bees in agro-ecosystems.- *Apidologie*, 41: 264-277.

DESNEUX N., DECOURTYE A., DELPUECH J.M., 2007.- The sub lethal effects of pesticides on beneficial arthropods.- *Annual Review of Entomology*, 52: 81-106.

DI PRISCO G., CAVALIERE V., ANNOSCIA D., VARRICCHIO P., CAPRIO E., NAZZI F., GARGIULO G., PENNACCHIO F., 2013.- Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees. In: *Proceedings of the National Academy of Sciences of the United States of America*, 110: 18466-18471.

DOUBLET V., LABARUSSIAS M., DE MIRANDA J. R., MORITZ R. F. A., PAXTON R. J., 2015.- Bees under stress: sublethal doses of a neonicotinoid pesticide and pathogens interact to elevate honey bee mortality across the life cycle.- *Environmental Microbiology*, 17: 969-983.

FLORIS I., 1991.- Dispersion index and sampling plans for honeybee (*Apis mellifera ligustica* Spin.) mite *Varroa jacobsoni* Oud.- *Apicoltura*, 7: 161-170.

FLORIS I., SATTA A., GARAU V. L., MELIS M., CABRAS P., ALOUL N., 2001.- Effectiveness, persistence, and residue of amitraz plastic strips in the apiary control of *Varroa destructor*.- *Apidologie*, 32: 577-585.

FLORIS I., SATTA A., CABRAS P., GARAU V. L., ANGIONI A., 2004.- Comparison between two thymol formulations in the control of *Varroa destructor* Anderson & Trueman: effectiveness, persistences and residues.- *Journal of Economic Entomology*, 97: 187-191.

FLORIS I., SATTA A., RUIU L., 2007.- Honeys of Sardinia (Italy).- *Journal of Apicultural Research*, 46: 198-209.

GAGGÌA F., BAFFONI L., STENICO V., ALBERONI D., BUGLIONE E., LILLI A., DI GIOIA D., PORRINI C., 2015.- Microbial investigation on honey bee larvae showing atypical symptoms of European foulbrood.- *Bulletin of Insectology*, 68: 321-327.

GENDE L., SATTA A., LIGIOS V., RUIU L., BUFFA F., FERNANDEZ N., CHURIO S., EGUARAS M., FIORI M., FLORIS I., 2011.- Searching for an American foulbrood early detection threshold by the determination of *Paenibacillus larvae* spore load in worker honey bees.- *Bulletin of Insectology*, 64: 229-233.

GENERSCH E., 2010.- American foulbrood in honeybees and its causative agent, *Paenibacillus larvae*.- *Journal of Invertebrate Pathology*, 103: S10-19.

GÓMEZ SAL A., GONZÁLEZ GARCÍA A., 2007.- A comprehensive assessment of multifunctional agricultural land-use systems in Spain using a multi-dimensional evaluative model.- *Agriculture, Ecosystems and Environment*, 120: 82-91.

HIGES M., MARTIN R., MEANA A., 2006.- *Nosema ceranae*, a new microsporidian parasite in honeybees in Europe.- *Journal of Invertebrate Pathology*, 92: 81-83.

HORNITZKY M. A. Z., KARLOVSKIS S., 1989.- A culture technique for the of *Bacillus larvae* in honeybees.- *Journal of Apicultural Research*, 28: 118-120.

KLEIJN D., BAQUERO R. A., CLOUGH Y., DIAZ M., DE ESTEBAN J., FERNANDEZ F., GABRIEL D., HERZOG F., HOLZSCHUH A., JOHL R., KNOP E., KRUESS A., MARSHALL E. J. P., STEFFAN-DEWENTER I., TSCHARNTKE T., VERHULST J., WEST T. M., YELA J. L., 2006.- Mixed biodiversity benefits of agri-environment schemes in five European countries.- *Ecology Letters*, 9: 243-254.

KREMEN C., WILLIAMS N. M., AIZEN M. A., GEMMILL-HERREN B., LEBUHN G., MINCKLEY R., PACKER L., POTTS S. G., ROULSTON T., STEFFAN-DEWENTER I., VAZQUEZ D. P., WINFREE R., ADAMS L., CRONE E. E., GREENLEAF S. S., KEITT T. H., KLEIN A. M., REGETZ J., RICKETTS T. H., 2007.- Pollination and other ecosystem services produced by mobile organisms: a conceptual framework for the effects of land-use change.- *Ecology Letters*, 10: 299-314.

LOUVEAUX J., MAURIZIO A., VORWOHL G., 1978.- Methods of melyssopalynology.- *Bee World*, 59: 139-157.

MAINI S., MEDRZYCKI P., PORRINI C., 2010.- The puzzle of honey bee losses: a brief review.- *Bulletin of Insectology*, 63: 153-160.

MARCHETTI S., 1985.- Il metodo dei sesti per la valutazione numerica degli adulti in famiglie di *Apis mellifera* L.- *Apicoltura*, 1: 41-61.

MILLENNIUM ECOSYSTEM ASSESSMENT, 2005.- *Ecosystems and human well-being: biodiversity synthesis*.- World Resources Institute, Washington, DC, USA.

NAZZI F., BROWN S. P., ANNOSCIA D., DEL PICCOLO F., DI PRISCO G.,VARRICCHIO P., VEDOVA G. D., CATTONARO F., CAPRIO E., PENNACCHIO F., 2012.- Synergistic parasite-pathogen interactions mediated by host immunity can drive the collapse of honeybee colonies.- *PLoS Pathogens*, 8 (6): e1002735.

OLEA L., SAN MIGUEL-AYANZ A., 2006.- The Spanish *dehesa*. A traditional Mediterranean silvopastoral system linking production and nature conservation, pp. 3-13. In: *Sustainable grassland productivity. Proceedings of the 21st general meeting of the European grassland federation*, 3-6 April 2006, Badajoz, Spain.

PERSANO ODDO L., RICCIARDELLI D'ALBORE G., 1989.- Melissopalynological nomenclature.- *Apicoltura*, 5: 63-72.

PERSANO ODDO L., SABATINI A. G., ACCORTI M., COLOMBO R., MARCAZZAN G. L., PIANA M. L., PIAZZA M. G., PULCINI P., 2000.- *I mieli uniflorali italiani nuove schede di caratterizzazione*.- Istituto Sperimentale per la Zoologia Agraria, Rome, Italy.

POTTS S. G., PETANIDOU T., ROBERTS S., O'TOOLE C., HULBERT A., WILLMER P., 2006.- Plant-pollinator biodiversity and pollination services in a complex Mediterranean landscape.- *Biological Conservation*, 129: 519-529.

QUARANTA M., AMBROSELLI S., BARRO P., BELLA S., CARINI A., CELLI G., COGOI P., COMBA I., COMOLI R., FELICIOLI A., FLORIS I., INTOPPA F., LONGO S., MAINI S., MANINO A., MAZZEO G., MEDRZYCKI P., NARDI E., NICCOLINI L., PALMIERI N., PATETTA A., PIATTI C., PIAZZA M. G., PINZAUTI M., PORPORATO M., PORRINI C., RICCIARDELLI D'ALBORE G., ROMAGNOLI F., RUIU L., SATTA A., ZANDIGIACOMO P., 2004.- Wild bees in agroecosystems and semi-natural landscapes. 1997-2000 collection period in Italy.- *Bulletin of Insectology*, 57: 11-61.

RATHCKE B. J., JULES E. S., 1993.- Habitat fragmentation and plant-pollinator interaction.- *Current Science*, 65: 273-277.

RICCIARDELLI D'ALBORE G., 1998.- *Mediterranean melissopalynology*.- Centro Stampa Università di Perugia, Italy.

ROSA A., TUBEROSO C. I. G., ATZERI A., MELIS M. P., BIFULCO E., DESSÌ M. A., 2011.- Antioxidant profile of strawberry tree honey and its marker homogentisic acid in several models of oxidative stress.- *Food Chemistry*, 129: 1045-1053.

RUTTNER F., 1986.- Geographical variability and classification, pp. 23-56. In: *Bee genetics and breeding* (RINDERER T. E., Ed.).- Academic Press, Orlando, USA.

SAN MIGUEL-AYANZ A., 2005.- Mediterranean European silvopastoral systems, pp. 36-40. In: *Silvopastoralism and sustainable land management* (MOSQUERA-LOSADA M. R., RIGUEIRO-RODRIGUEZ A., MCADAM J., Eds).- CABI Publishing, Wallingford, UK.

SATTA A., FLORIS I., EGUARAS M., CABRAS P., GARAU V. L., MELIS M., 2005.- Formic acid-based treatments for control of *Varroa destructor* in a Mediterranean area.- *Journal of Economic Entomology*, 98: 267-273.

SHIMANUKI H., CALDERONE N. W., KNOX D. A., 1994.- Parasitic mite syndrome - the symptoms.- *American Bee Journal*, 134: 827-828.

STEFFAN-DEWENTER I., KUHN A., 2003.- Honeybee foraging in differentially structured landscapes.- *Proceedings of the Royal Society - Biological Sciences*, 270: 569-575.

STEFFAN-DEWENTER I., WESTPHAL C., 2008.- The interplay of pollinator diversity, pollination services and landscape change.- *Journal of Applied Ecology*, 45: 737-741.

TSCHARNTKE T., KLEIN A. M., KRUESS A., STEFFAN-DEWENTER I., THIES C., 2005.- Landscape perspectives on agricultural intensification and biodiversity - ecosystem service management.- *Ecology Letters*, 8: 857-874.

VANENGELSDORP D., MEIXNER M. D., 2010.- A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them.- *Journal of Invertebrate Pathology*, 103: S80-S95.

VANENGELSDORP D., SPEYBROECK N., EVANS J. D., NGUYEN B. K., MULLIN C., FRAZIER M., FRAZIER J., COX-FOSTER D., CHEN Y. P., TARPY D. R., HAUBRUGE E., PETTIS J. S., SAEGERMAN C., 2010.- Weighing risk factors associated with bee colony collapse disorder by classification and regression tree analysis.- *Journal of Economic Entomology*, 103: 1517-1523.

VILLANUEVA-GUTIERREZ R., 2002.- Polliniferous plants and foraging strategies of *Apis mellifera* (Hymenoptera: Apidae) in the Yucatán Peninsula, Mexico.- *Revista de Biologia Tropical*, 50 (3-4): 1035-1044.

VON DER OHE W., PERSANO ODDO L., PIANA L., MORLOT M., MARTIN P., 2004.- Harmonized methods of melissopalynology.- *Apidologie*, 35: 18-25.

Authors' addresses: Alberto SATTA (corresponding author, albsatta@uniss.it), Ignazio FLORIS, Luca RUIU, Franco BUFFA, Dipartimento di Agraria, sezione di Patologia vegetale ed Entomologia, Università di Sassari, viale Italia 39, 07100 Sassari, Italy; Simonetta BAGELLA, Maria Carmela CARIA, Dipartimento di Scienze della Natura e del Territorio, Università di Sassari, via Enrico De Nicola, 07100 Sassari, Italy.

Received June 26, 2015. Accepted December 3, 2015.