Beekeeping in Romania and artificial insemination of honey bee, *Apis mellifera*. State of the art

[1]Remus O. Stoian, [2] Miklos Botha, [3,4]I. Valentin Petrescu-Mag

[1] University of Oradea, Doctoral School of Engineering, Oradea, Romania; [2] SC Bioflux SRL, Cluj-Napoca, Romania; [3] University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Agriculture, Cluj-Napoca, Romania; [4] University of Oradea, Doctoral School of Engineering, Oradea, Romania. Corresponding author: R. O. Stoian, remusoctav@yahoo.com

Abstact. The aim of this study is to draft a scientific summary comprehensive enough to serve as a starting point in designing the experimental plan whose objective will be to improve the instrumental insemination technique in bees (*Apis mellifera*). In Romania, due to the pedo-climatic and environmental conditions, the selection, improvement and maintenance in pure state of the selected genetic material may be conducted in a 100% controlled manner by instrumental insemination. The published literature mentions that in order to reduce the risk of sperm and queen contamination with pathogenic agents it is recommended that the drones eliminate the fecal matters before collecting sperm from them. However, it is not specified how this is performed. Moreover, the literature does not mention whether the queens are allowed to fly before the insemination. In a future study we will investigate the importance of drones and queens dejection before performing the insemination procedure, as we presume that there is a real contamination risk of the seminal material, of the tools and implicitly of the queens with fecal matters, which can compromise the result of instrumental insemination.

Key Words: instrumental insemination, artificial reproduction, honey bee, defecation.

Introduction. The aim of this study is to draft a scientific summary comprehensive enough to serve as a starting point in designing the experimental plan whose objective will be to improve the instrumental insemination technique in bees (*Apis mellifera*).

1. Beekeeping nowadays: achievements and perspectives

Beekeeping is interrelated today with biotechnologies, science of reproduction, genetics, entomology, ethology, biochemistry, medicine, biodiversity (Mărghitaş 2008); all these sciences and information are used to understand the phenomena that set the basis for the enhancement of bees health and honey production (Mattila & Seeley 2007; Goblirsch 2017). At the moment, the selection of valuable specimens is no longer done empirically, but based on molecular biology and experimental statistics criteria. Productivity, the reproductive abilities and the bees health are associated to some nucleotide sequences, proteins, behavioral sequences, with the morphology of the insect body, etc., so that the approach of the above mentioned aspects becomes a very complex one (Crozier & Crozier 1993; Evans et al 2006; Estoup et al 1993, 1995; Garnery et al 1992).

Nowaday, the bee is not only a production animal of strictly zootechnical use (Odagiu & Oroian 2010), it is an excellent bioindicator (Celli & Maccagnani 2003), a model organism for the monitoring of biodiversity (Mag et al 2006), for sexuality and determinism of the sexes (Baer 2005), for parasitology (Ball & Allen 1980; Murilhas 2002; Rosenkranz 1999), a model for the behavioral studies (Page 1980; Beekman & Ratnieks 2000), especially since we are dealing with the behavior of the individual, to which we may add the behavior of the bee hive operating as a super-organism (Moritz & Southwick 2012). The bee is important for the understanding of communication between animals, for the understanding of pheromonal and hormonal reactions involved in communication and behavior (Schneider & Lewis 2004).

The honeybee is an organism proposed by Cridge et al (2017) to be a model insect for the understanding of the organism development genetics and of early development plasticity. Further on, in the adult phase, it is interesting to understand the plasticity of ovary development depending on the necessities of the colony at a certain time (Cridge et al 2017). All these intriguing aspects are based on biochemical, physiological, genetic and molecular explanations which us, the scientists, hope to discover, but not in this paper.

1.1. Evolution of beekeeping in Romania

In our country beekeeping has a millennial tradition, as favorable natural, climatic and pedological conditions and rich honey resources have determined the spread of the bees in the entire country area since very old times (Cornoiu 2012; Mărghitaş 2008). The start of the beekeeping practice is difficult to identify because of the lack of written sources at that time. It is certain, however, that for a long time the main product of this activity, the honey, was used as food and sugar substitute by those who, in the beginning, gathered it from the tree cavities in forest populated by bees (Zeuner 1963). As humans learn fast, the transition from this honey gathering to the removal of bees cavities from the forest and their settling near man's house, did not take long. And from this beginning to the arrangement and then building of primitive beehives from withs, straws, reed, branches, clay or other materials, was yet another small step (Mason & Mason 1984). Therefore, from a simple honey gatherer, man has gradually become a beekeeper (Manualul Apicultorului (The Beekeeper's Manual), 9th edition, 2007, p.12). Although the beekeeping in the earliest periods was lacking the detailed scientific and technical information, it has evolved over generations to be the successful economic branch it is today.

The antique writings of the historian Herodot (years 485 – 425 B.C.) or of the known ancient naturalist Claudius Aelianus (3rd century B.C.), speak about the bees on the territory of Dacia and Tracia.

Also, there are still many historical, archaeological, linguistic, ethnographic and folkloric data proving unequivocally the ongoing preoccupation and continuity of beekeeping in the Carpato-Danubiano-Pontic space (Manualul Apicultorului (The Beekeeper's Manual), 9th edition, 2007, p.12). For example, the petrified honeycombs discovered in a rivulet in Siliște, Cluj county, as well as the wax tablets used by the Romans for writing, found in an old mine in Roșia Montană, in the Apuseni Mountains, represent undisputed proof that bees existed in our country since ancient times (Manualul Apicultorului (The Beekeeper's Manual), 9th edition, 2007, p.12). On certain monuments, including the column of Traian in Rome, there are pictured scenes of bees, which prove, among others, the evolved development of the beekeeping activity among the geto-dacic population at that time. The bees are also present in the Romanian folklore, in documents proving different transactions, donations, inheritances, etc (Tudor 2011).

After the forming of the Romanian people, the Romanian and foreign historians wrote about the beekeeping activity in our country and about the measures taken for the circulation of products, as well as about the prosperity of beekeeping in Muntenia and Moldova. Among these historians we mention the lord of Moldova, Dimitrie Cantemir (1717), Peyssonel (1781), Ion Neculce and Grigore Ureche. Other sources referring to the past beekeeping activities are the princely documents (papers cited in Manualul Apicultorului (The Beekeeper's Manual) 2007).

With the occurrence of beekeeping as science, the first detailed writings on beekeeping are produced. The first Romanian book on beekeeping, entitled The Economy of Hives (Economia stupilor) and published in 1785, belongs to the ophthalmologist and bookman Ioan Piuariu-Molnar. Subsequently, in the year 1823, the professor priest Ioan Tomici published a book on beekeeping entitled The Culture of Bees (Cultura albinelor), edited in Buda and printed in Cyrillic letters at the Crăiasca University in Pesta (Manualul Apicultorului (The Beekeeper's Manual) 2007).

The year 1873 remained in the history of the Romanian beekeeping activity as a mark of the first intent to organize the beekeepers in a structural and functional form to protect their interests. Their organization protected the beekeepers and provided the opportunity for further development by founding the beekeeping association in Buziaş, entitled The Reunion of Beekeepers in Banat („Reuniunea Apicultorilor Bănățeni"). The president of this association was Adam Petru Jager, and its secretary was the primary school teacher Nicolae Grand (Manualul Apicultorului (The Beekeeper's Manual) 2007)

The Beekeeper's Association („Asociația Crescătorilor de Albine") was created in 1957. So far, this association achieved remarkable results in promoting beekeeping and honeybee products. As a result of its affiliation to international entities in beekeeping purposes, Romania was able to conduct cooperation relations and experience exchange with other beekeepers in the world. In Bucharest, in the year 1965, it was organized the 20th International Congress of the International Beekeeping Federation Apimondia (headquarters in Rome) (Tudor 2011). On this occasion, the Romanian professor V. Harnaj became the president of the International Federation of Beekeeping Associations Apimondia. He filled this position since 1965 until 1985, contributing significantly to the development of Romanian beekeeping activity (Manualul Apicultorului (The Beekeeper's Manual) 2007).

Romanian beekeeping has enhanced its professional statute over the years, among the countries with acknowledged tradition in this activity. After the revolution in 1989, Romania underwent significant decreases of the number of beekeepers, of the bee hives and of the zonal influence of our country in the bee trade (Bucată 2001a). Over the last two decades there was a more and more evident trend to make the transition from amateurish approach to professional beekeeping. The increase in economic motivation of the transition to industrial beekeeping should be further supported and encouraged by various regulations of the subsidy type. The small, 50-70 bee hives apiaries, representing the minimum level defining an apiary, generate productions which usually go directly to consumers (Bucată 2001a).

1.2. The spread of the bees around the globe

Bees are spread on the entire globe, except in the most elevated altitudes, the two polar regions and a few small oceanic isles (Mason & Mason 1984). If, in the high or polar regions the absence of the bees is explained by temperatures too low or by the lack of floristic richness (Winston 1991), in the case of insects, the geographical remoteness, lack of soil or powerful currents may be considered the main obstacles for their spreading. The largest diversity of the bee species is found in the warm, arid or semiarid regions in Asia, South America and Mexico, with a hotspot of biodiversity in Borneo (Koeniger et al 2010).

1.3. Taxonomy

1.3.1. Classification of the species

Kingdom: Animalia (eukaryotic and pluricellular, generally heterotrophic organisms)
Subkingdom: Eumetazoa (animals without spine and without internal bone system)
Phylum: Arthropoda (invertebrate animals with articulated legs)
Subphylum: Mandibulata (arthropods provided with mandibles)
Class: Insecta (arthropods with the body composed generally of three distinct segments: head, thorax and abdomen; exceptions from the presence of the three segments are caused by subsequent adaptations)
Subclass: Pterigota (insects that have two or three pairs of wings on the thorax segments)
Order: Hymenoptera (insects provided with wings of membranous consistency)
Suborder: Apocrita (hymenoptera where the connection between thorax and abdomen is done by a thin portion, called stalk)
Group: Aculeata (insects provided with stinger)
Suprafamily: Apoidea (insects who feed their brood with pollen and flower nectar)
Family: Apidae
Subfamily: Apinae (insects who build nests and have a pollen collecting apparatus at the third pair of legs)
Tribe: Apini

Genus: *Apis* (bees that survive in permanent or monogin hives - they have only one female, called queen - with developed breeding organs to ensure perpetuation; their body is covered with short and rare root hair)
Species:
A. dorsata (giant Indian bee, it builds one honeycomb hanging on rocks or branches of various trees; it is the largest bee known and it is spread in India, south of China, the Indonesian archipelago and the Philippines);
A. florea (dwarf Indian bee, the smallest bee known, it builds only one very small honeycombs hanging on the branches of trees; it is spread in Malaysia, India, Borneo and Jawa);
A. cerana (also called the ordinary Indian bee, the nest is built in cavities and includes multiple honeycombs; it is spread in China, Japan, India, Indonesia, Jawa, Borneo, Sumatra, but also in Russia - The Far East);
A. mellifera (the honeybee, very well known and the most common bee, used by man for its productive qualities; its nest is built in closed cavities, on multiple honeycombs, with a large number of individuals per hive. The name of the species, *mellifera* was proposed by Linne in 1758 and it was lated changed (1761) into *mellifica;* in the end the previous *mellifera* name was adopted.

1.3.2. Infraspecific taxa of the *A. mellifera* species

1.3.2.1. ***Subspecies***

According to the current zoology and taxonomy ("the evolutionary species concept" - Simpson 1961; Wiley & Mayden 2000; Kottelat & Freyhof 2007; Nowak et al 2009), subspecies have disappeared from the nomenclature, being raised at species level, either lowered at variety or breed level (Batin & Roman 2016). "The evolutionary species concept" was suggested by Simpson (1961) (and reconsidered by Wiley 1978) to adapt the concept of biological species to the paleontological context: a species is an evolutionary line (a sequence of ancestor and descendant populations) which evolves separately from other lines, having its own evolutionary roles and unitary trends.

However, the beekeeper entomologists did not agree on the new trend, that is why some bee groups still appear either as breeds or as subspecies of the honeybee *Apis mellifera.* Thus, as we are not in the position to bring order to the taxonomy of insects; we will address them according to the late published literature.

1.3.2.1.1. ***Subspecies originating in Europe***

Apis mellifera ligustica Spinola 1806, Italian bee. It is the most common breed in North America, South America and the southern part of Europe. The Italian bee is bred in almost the entire world for commercial purposes. It is a familiar subspecies and it is not highly predisposed towards any swarming, producing a consistent honey surplus. The Italian bees have few undesirable characteristics, but colonies tend to keep large populations throughout the cold season and that is why they need more food resources in order to survive the winter as compared to other subspecies in the temperate zones.
Apis mellifera carnica Pollmann 1879*,* the Carniolan bee. Carniola is a region in Slovenia, in the southern part of the Austrian Alps, in the northern part of the Balkans. This subspecies is popular to the breeders due to its docility, as it is easy to work in the apiary populated by such bees.
Apis mellifera caucasia Pollmann 1889, the Caucasian bee (Georgia).
Apis mellifera remipes Gerstäcker, 1862, located predominantly in Iran, the Caspian Sea.
Apis mellifera mellifera Linnaeus, 1758, the European bee (Northern and Western Europe). It is one of the most frequently bred bee breed.
Apis mellifera iberiensis Engel, 1999.
Apis mellifera cecropia Kiesenwetter, 1860, lives in the south of Greece.
Apis mellifera cypria Pollmann, 1879.
Apis mellifera ruttneri Sheppard, Arias, Grech & Meixner 1997, it is a subspecies originating in the Maltese Islands.
Apis mellifera sicula Montagano, 1911.

Apis mellifera carpatica – the Romanian bee, addressed either as a subspecies, or as a breed, it has been recently characterized and approved by a team under the supervision of ANZ (Romania). The breed/subspecies does not strictly occupy the area of our country and underwent hybridizations with other subspecies/breeds over time.

1.3.2.1.2. Subspecies originating in the African continent

Apis mellifera scutellata Lepeletier, 1836.
Apis mellifera capensis Eschscholtz, 1822.
Apis mellifera monticola Smith, 1961.
Apis mellifera sahariensis Baldensperger, 1932.
Apis mellifera intermissa von Buttel-Reepen, 1906.
Apis mellifera major Ruttner, 1978.
Apis mellifera adamsonii Latreille, 1804.
Apis mellifera unicolor Latreille, 1804, lives in Madagascar.
Apis mellifera lamarckii, Cockerell, 1906.
Apis mellifera litorea Smith, 1961.
Apis mellifera nubica Ruttner, 1976, populates areas of Sudan.
Apis mellifera jemenitica Ruttner, 1976, populates biotopes in Somalia, Uganda, Sudan and Yemen.

1.3.2.1.3. Subspecies originating in the Middle East and Asia

Apis mellifera macedonica Ruttner, 1988, populates regions in the Republic of Macedonia and the north of Greece.
Apis mellifera meda Skorikov, 1829, populates areas in Irak.
Apis mellifera adamii Ruttner, 1977, lives in the island of Crete.
Apis mellifera armeniaca, (possibly synonym with *Apis mellifera remipes*), identified as living in the Middle East, Caucasus, Armenia.
Apis mellifera anatoliaca Maa, 1953. This breed is typical to the colonies in the central region of Anatolia in Turkey and Irak (it is spread in the west to Armenia). It has many useful characteristics, but sometimes the work inside and outside the hive may be unpleasant.
Apis mellifera syriaca Skorikov, 1829, the bee in Syria, lives in the Near East and Israel.
Apis mellifera pomonella Sheppard & Meixner, 2003. These are the endemic honeybees in the Tien Mountains (Central Asia). This subspecies is spread to the farthest East.

1.4. Bred breeds/subspecies

Nowadays, as a result of human intervention, *Apis mellifera* (the bee) can be found from the Arctic Circle to the Cape of Good Hope. With the globalization process, man has introduced in all geographical areas where climate was permissive, the most productive subspecies/breeds of bees. The main breeds of bees used by the professional beekeepers are those listed in Table 1.

Table 1

The main subspecies/breeds of bees used by the professional beekeepers

Breed/subspecies	*Origin*	*Tube length*	*Color*
Apis mellifera mellifera (the European bee)	Western and Northern Europe	6.05-6.35	Black
Apis mellifera ligustica	Italy	6.50	Yellow
Apis mellifera caucasia	Georgia	7.20	Grey to beige
Apis mellifera carnica	Pen. Balkan	6.60	Grey
Apis mellifera adamsonii	Sub-Saharan Africa	5.68	Ruddy yellow
Apis mellifera iberiensis	Spain, south of the Barcelona – Burgos line	6.44	Black

One colony of honeybees is organized in castes; it is composed of up to 50 thousand worker bees, a few thousands of seasonal drones and one queen which produces eggs and is, generally speaking, the mother of all individuals in the hive (Winston 1987).

1.5. Honeybee market

The European Union (EU) is the second honey producer in the world (Table 4), after China. The total number of hives in the EU is approx. 17 million, owned by approx. 600.000 beekeepers (European Commission, 2018). However, EU does not produce enough honey products to cover its own consumption needs. EU imports significant amounts of honey from China (approx. 40% of the total honey imports) (European Commission, 2018). Romania is the most important honey producer in the European Union (Figure 1), although it has a smaller area, and a smaller number of hives (Table 2, Figure 2) and beekeepers (Table 3, Figure 3) as compared to Spain or other top countries.

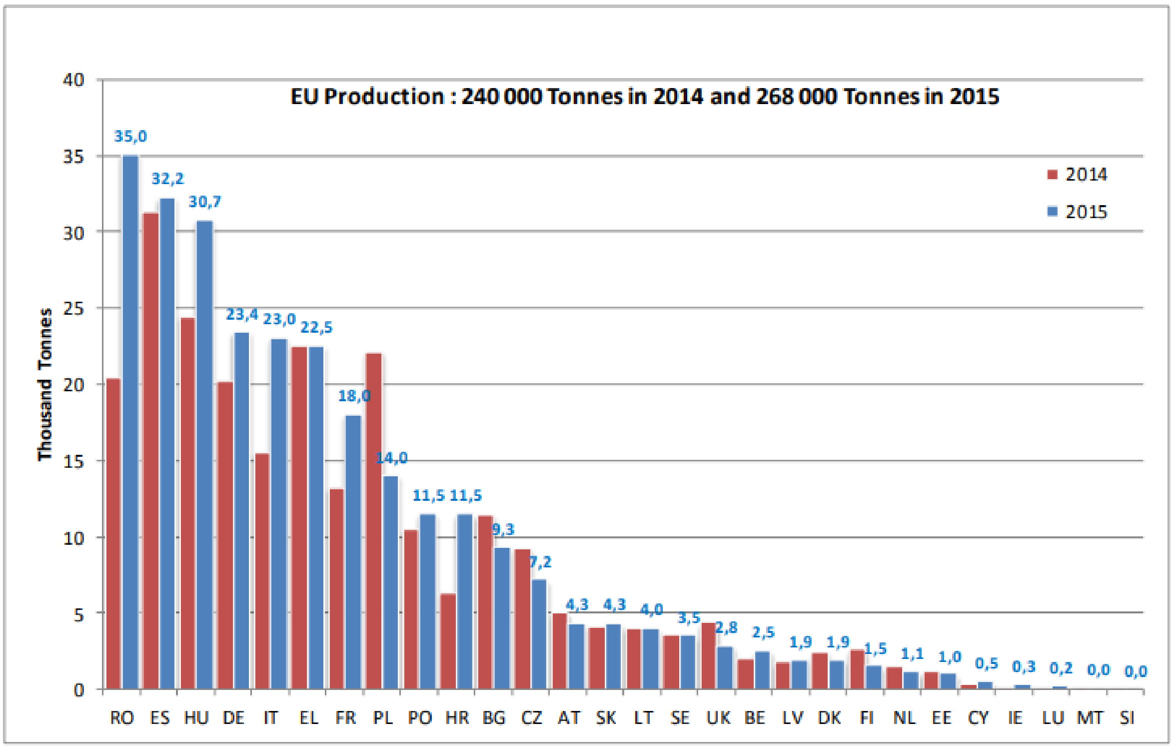

Figure 1. Honey bee production in the EU (European Commission 2018).

Table 2

Evolution of the total number of bee hives in the EU (European Commission 2018)

Country	*2003*	*2004-2006*	*2008-2010*	*2011-2013*	*2014-2016*	*2016*	*Obs.*
ES	2,398	2,465	2,321	2,459	2,459	2834	+15.2%
RO	-	-	975	1,280	1,550	1,472	+59.5%
PL	-	949	1,092	1,123	1,281	1,505	+17.5%
IT	1,100	1,100	1,157	1,128	1,317	1,356	+3.0%
Fr	1,297	1,150	1,361	1,339	1,636	1,322	-19.2%
EL	1,380	1,388	1,468	1,502	1,584	1,248	-21.2%
HU	-	873	900	900	1,089	1,184	+8.8%
DE	90	890	751	712	711	807	+13.5%
BG	-	-	672	617	526	754	+43.4%
PT	633	590	555	563	567	700	+23.5%
CZ	-	478	526	498	541	671	+24.1%

Country	*2003*	*2004-2006*	*2008-2010*	*2011-2013*	*2014-2016*	*2016*	*Obs.*
HR	-	-	-	-	492	406	-17.5%
AT	344	327	311	368	376	354	-6.0%
SK	-	192	246	236	255	292	+14.6%
UK	274	274	274	274	274	223	-18.5%
LT	-	84	85	118	145	186	+28.4%
SI	-	143	171	143	167	181	+8.7%
SE	145	145	150	150	150	154	+2.7%
DK	155	160	170	170	150	115	-23.1%
LV	-	54	62	64	84	97	+15.4%
NL	80	80	80	80	80	79	-1.1%
BE	100	111	110	112	108	65	-39.6%
FI	42	47	56	46	50	64	+28.0%
EE	-	51	33	25	41	44	+7.4%
CY	-	46	44	44	45	43	-4.0%
IE	20	20	22	24	16	21	+36.2%
LU	10	11	9	8	8	6	-27.4%
MT	-	2	2	2	2	4	+22.1%
EU 15/25/27/28	8,877	11,631	13,603	13,985	15,704	17,189	+9.5%

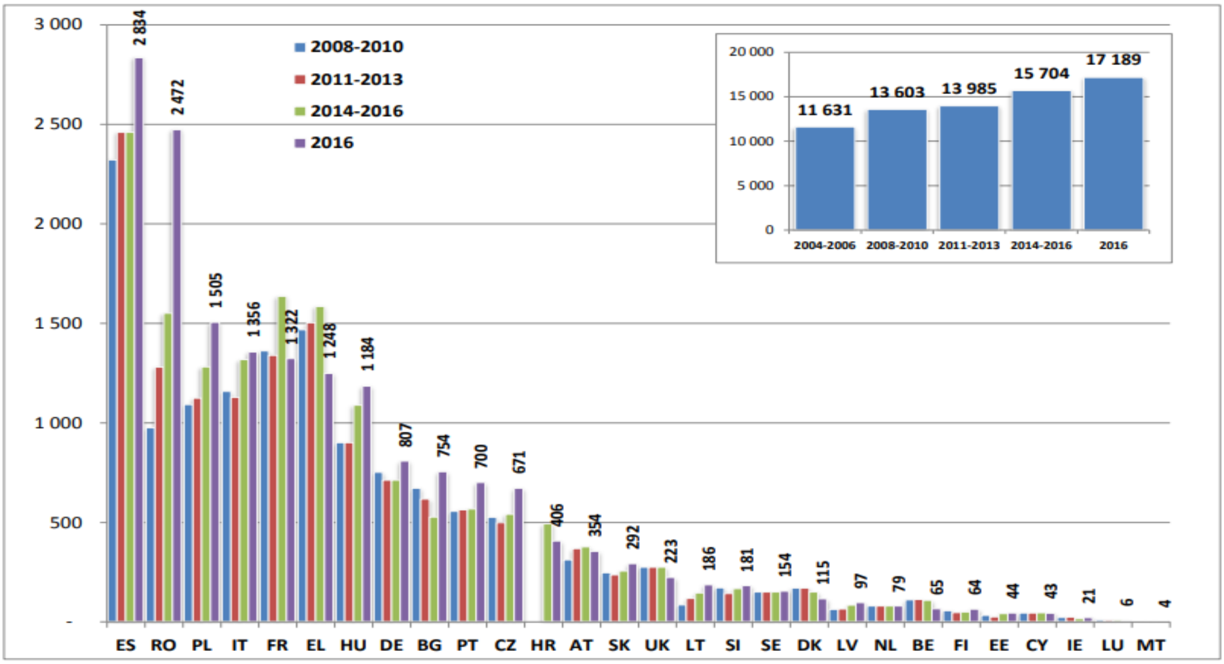

Figure 2. Total number of hives in EU, expressed in thousands (European Commission 2018).

Table 3

Evolution of the total number of beekeepers in the EU (European Commission 2018)

Country	*2003*	*2004-2006*	*2008-2010*	*2011-2013*	*2014-2016*	*2017-2019*
DE	103,600	103,600	103,600	103,600	98,297	116,000
PL	-	42,800	39,410	44,999	51,778	62,575
IT	75,000	50,000	70,000	70,000	50,000	50,000
CZ	-	49,734	48,678	46,033	48,132	49,486
FR	100,000	10,000	69,600	73,500	75,000	41,560
UK	43,600	43,600	43,900	43,900	43,900	37,888
AT	25,027	24,421	23,000	24,451	25,099	25,277
EL	22.000	19,560	19,814	19,392	21,031	24,582
ES	27,420	24,606	23,265	23,816	23,473	23,816
RO	-	-	36,800	40,000	43,200	22,930
HU	-	15,302	16,000	16,000	20,410	21,565
BG	-	-	29,097	29,097	19,179	17,969
SK	-	18,123	14,339	14,699	16,338	17,171
SE	14,000	14,000	15,000	15,000	12,000	14,000
HR	-	-	-	-	8,953	12,526
PO	26,000	22,000	15,267	17,291	16,774	10,698
SI	-	7,955	7,620	8,838	9,638	10,145
BE	11,000	7,715	7,000	7,600	9,500	9,490
LT	-	11,000	10,923	13,000	10,132	8,536
DK	6,000	5,000	4,278	4,300	5,500	7,000
NL	10,000	10,000	10,000	8,000	8,000	7,000
EE	-	7,600	7,400	2,416	5,934	5,250
LV	-	8,300	3,300	3,700	3,346	3,282
FI	4,200	4,200	3,300	2,500	2,700	3,100
IE	2,300	2,200	2,200	2,388	2,326	3,000
CY	-	632	544	588	550	691
LU	650	650	369	348	331	337
MT	-	168	168	182	215	208
EU	470,797	593,168	624,872	635,638	631,236	606,082

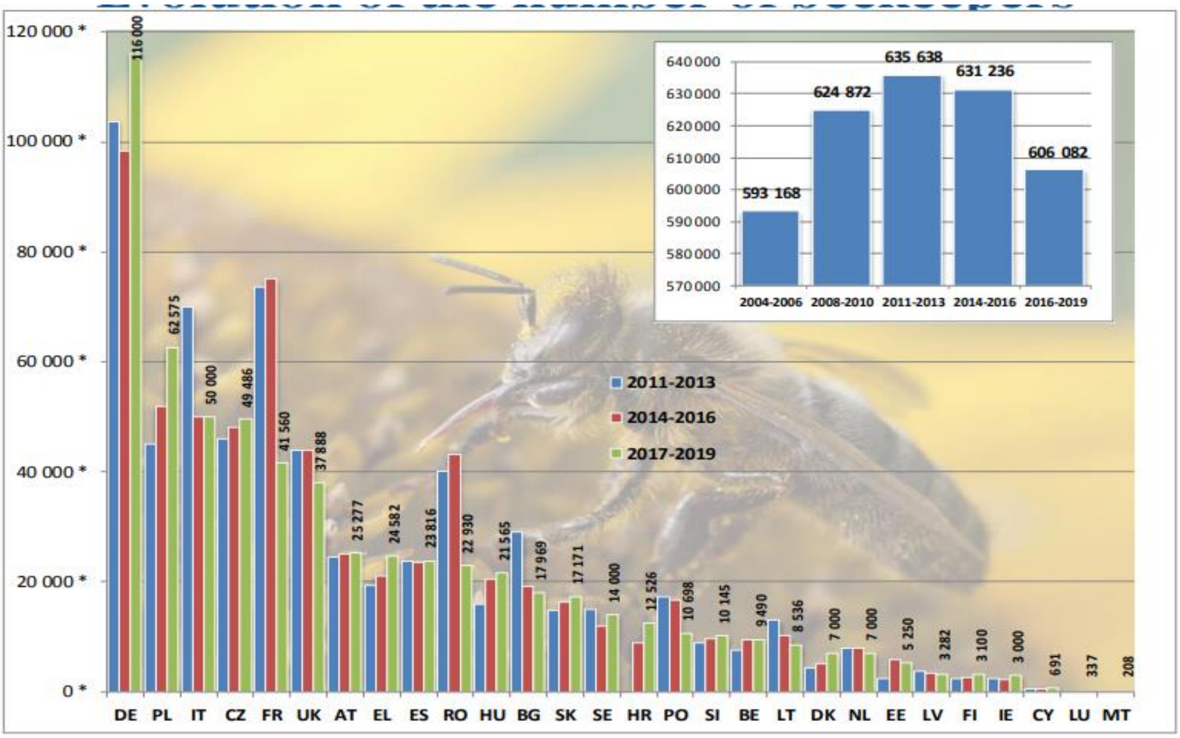

Figure 3. Evolution of the total number of beekeepers in the EU (European Commission 2018).

Table 4

World honey bee production per country in thousands of tones (European Commission 2018)

Specification	*2001*	*2002*	*2003*	*2004*	*2005*	*2006*	*2007*	*2008*	*2009*	*2010*	*2011*	*2012*	*2013*	*2014*
China	254	268	295	298	300	338	358	407	407	409	446	462	463	474
EU	182	178	200	187	182	191	195	189	194	180	225	195	212	161
Turkey	60	75	70	74	82	84	74	81	82	81	94	89	95	104
USA	84	78	82	83	73	70	67	74	66	80	67	65	68	81
Iran	27	28	15	29	35	36	47	41	46	51	51	71	75	76
Russia	53	49	48	53	52	56	54	57	54	52	60	65	68	75
Ukraine	60	51	54	58	71	76	68	75	74	71	70	70	74	67
India	52	52	52	52	52	52	51	55	55	60	60	60	61	62
Mexico	59	59	57	57	51	56	55	60	56	56	58	59	57	61
Brazil	22	24	30	32	34	36	35	38	39	38	42	34	35	38
Canada	35	37	35	34	36	48	31	29	32	37	36	41	35	37
Tanzania	27	27	27	28	28	28	28	27	28	29	28	29	30	31
Angola	24	23	23	23	24	23	23	23	23	23	23	23	23	23
Republic of Korea	22	20	18	16	24	23	26	26	28	23	18	22	22	23
Uruguay	10	10	10	13	10	13	16	10	8	15	19	20	20	20
New Zealand	9	5	12	9	10	10	10	12	13	13	9	10	18	18
Viet Nam	7	11	13	11	14	17	16	10	12	12	12	12	13	14
Australia	19	18	16	17	17	18	18	18	16	15	10	12	14	13
Bulgaria	5	5	9	8	11	10	6	11	10	11	10	9	10	9
Uzbekistan	2	3	2	2	2	2	2	3	3	3	4	5	7	9
Thailand	4	4	4	4	5	4	8	8	8	8	8	8	9	9
Cuba	7	6	7	6	4	7	6	5	5	5	7	7	7	8
Others	195	231	228	236	171	284	259	237	228	230	257	291	265	99
Total	1,219	1,262	1,305	1,329	1,387	1,482	1,452	1,497	1,486	1,499	1,614	1,880	1,678	1,511

2. The place of beekeeping among agricultural sciences: at the border between agronomy and animal science

The bee is a component of the animal kingdom with numerous references to zoology and it is mainly studied by zoologists. That is why beekeeping is arbitrarily placed by most of the scientists in the zoological domain. However, the honeybee products are considered predominantly vegetal products (Costea & Stroia 2011). The pollen, the bioactive compounds and the plant saccharides from honeybee products are the argument of integrating beekeeping in the domain of food science or agronomy in the broad sense (Dezmirean et al 2012; Bucată 2001b). Anyway, both the agronomists and the nutritionists, and the zootechnical engineers study bees and honeybee products, and their combined efforts by interdisciplinary approach bring new information which are translated into scientific and technological progress. In many initiatives, the floristic biodiversity is approached by the analysis of pollen diversity from the honey composition (Mag et al 2006), bioecology and beekeeping technique going hand in hand (Lazăr 2002; Tanasoiu et al 2015; Bura et al 2003).

3. Honey bee reproduction, artificial insemination and the state of the art

Without claiming that we can treat honey bee reproduction exhaustively and everything it has been studied about it so far, we will try to present broadly what we have studied about it until now. We will try to present the state of the art in the research conducted by Romanian and foreign beekeepers, as a starting point for our doctoral studies.

3.1. Reproductive apparatus

The genital apparatus of the bee is composed differently in the two genders. It is composed first of the sexual gland, testicle for the male (called drone) and ovary for the female (called queen), organs which produce the specific sexual cells – spermatozoa in the drone and egg cells in the queen. The genital apparatus is composed of the sexual gland, genital tract and accessory glands (Snodgrass 1984; Aioanei & Stavrescu-Bedivan 2011).

3.1.1. Male reproductive apparatus

Starting in order from the inside towards the outside, it is composed of: testicles, vasa deferentia, seminal vesicles, mucous glands, ejaculatory duct and penis (Snodgrass 1984) (Figure 4).

The testicles are located in the abdomen cavity, between the digestive tube and the cord, being fixed to the abdomen by two ligaments. The testicles are yellowish, have a heterogeneous aspect, on the outside they are wrapped in a tunic, while on the inside they contain approx. 200 sperm producing tubes (called testioles) which open at the end of the vas deferens into a joint room. The climax of the testicles development takes place in the larvae stage, in the adult stage the sizes of the testicles are significantly reduced (Snodgrass 1925).

The next component part of the male reproductive apparatus is represented by the vasa deferentia. The vasa deferentia are miniature tubes by which the seminal material produced in the testicles reaches the seminal vesicles. Therefore, the vasa deferentia are tubular connection formations.

The seminal vesicle is the thickened portion of the vasa deferentia, with the role of collecting and keeping the sperm. The seminal vesicle finishes in a short duct which opens on the back side of the mucous gland, at the base of this gland. The wall of the vas deferens and of the mucous gland has strong bi- or three-layered muscles. In the part towards the lumen, the wall is formed of a layer of tall secretory epithelial cells. While in the seminal vesicle these cells produce a fluid with nutritive properties and suspension role for spermatozoa, in the mucous gland the epithelial cells produce mucus (Snodgrass 1925, 1984).

The mucous glands are accessory elements of the reproductive apparatus and have the shape of some bags which open at the junction of the seminal vesicles and the ejaculatory duct. The secretion product of the mucous glands has a slightly basic ph and, in contact with water or air, it agglutinates. Together with the secretion of seminal vesicles it forms the spermatic fluid. The mucus produced by these glands has the role of diluting the sperm, thus facilitating its elimination when ejaculating (Snodgrass 1925).

The next component part of the male bee genital apparatus is the ejaculatory duct. This has the shape of a long and thin tube, without muscles, which unites the gland edges with the anterior edge of the phallus. The ejaculatory duct communicates with the glands at the moment of mating due to the action of the glandular muscles (Dade 1994).

The terminal part of the genital apparatus in the drone is represented by the endophallus (or penis). The penis is found in the ventral part of the abdomen and, previously, it goes until the third segment of the abdomen. This is the copulatory organ, it is voluminous and it is composed of the following (Snodgrass 1984):

- penis vestibule, which opens to the outside by a phallotrema;
- The horns and the cervix, two formations similar to some wide and sharped-edges bags; the cervix is provided with small spicules, such as the vestibule;
- The penian bulb is the terminal, swollen part of the penis into which the ejaculatory duct opens.

This has thin walls, smooth on the inside and on the dorsal side, having two chitinous consistency, hard, long plates, directed with their tips towards the posterior side; it is full of a glandular secretion mass and spermatozoa and it opens in the cervix by a triangle shape orifice (Woyke 2008; Snodgrass 1984).

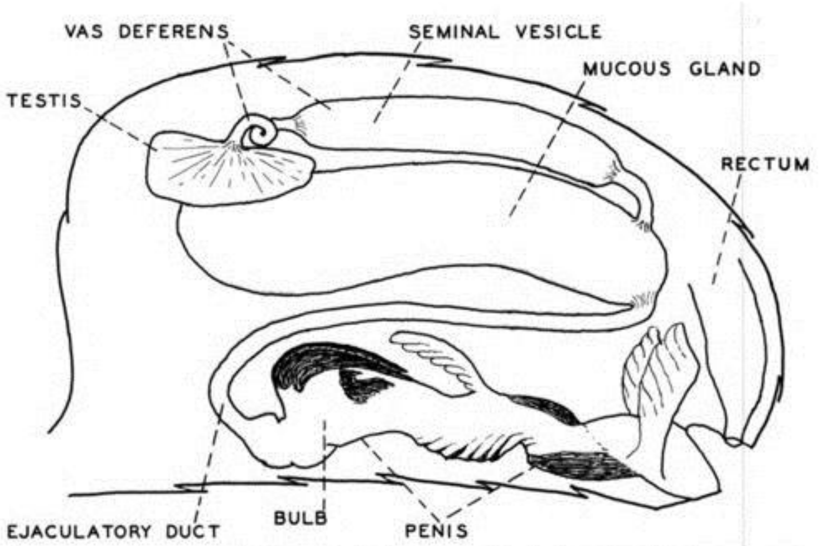

Figure 4. The male reproductive apparatus (Mackensen & Tucker 1970).

3.1.2. Female reproductive apparatus

The queen’s reproductive apparatus is located in the abdominal cavity, occupying a very large space in the fecundated queen. Similar to the male, the female reproductive apparatus has several component parts; in this case it is composed of ovaries, even oviducts, odd oviduct, spermatic chamber and vagina (Snodgrass 1984) (Figure 5).

The ovaries are two voluminous, pear-shaped organs, located at the upper part of the abdomen, more precisely above the goiter. These are composed each of 150-180 egg tubes (ovariole) in the queen and of 2 up to 12 egg tubes in the worker bee. Throughout their entire length there can be noticed strangulations corresponding to the ova in various development stages: at the top of the egg tube there are the ovogonia, then the ovocytes, and in the terminal part the egg cells. In the reproductive season, for an egg set of approx. 1500 eggs a day, each ovariole produces approx. 4-5 eggs.

The even oviducts and the odd oviduct make the connection between the ovaries and the vagina and play the role in eliminating the egg cells. In the queen, the above mentioned oviducts are located in the shape of the letter Y and are different from one another from the histological point of view.

The even oviducts have thin walls, composed only of a very weakly developed fascicle of one-layered muscle fibers which represent small bags with folds directed length-wise, which enable the significant increase of the lumen volume when needed. In the young queen, the oviducts are very elongated, and the ovaries are small in size, but after the beginning of the egg laying, the oviducts become shorter as the ovaries increase their size (Winston 1991).

By contrast with the even oviducts, the odd oviduct is provided with very well developed muscles, and its cross segment (which has an anterior opening of 0.33 mm) cannot be widened almost at all. For this reason, the egg (with the diameter of 0.39-0.42 mm) becomes ellipsoidal in shape when passing through this oviduct, due to compression.

The spermatheca is located above the odd oviduct and the vagina, respectively, with the vulnerant apparatus (the insect's sting) on the front side. It represents the reservoir for sperm storage with a diameter of 1.2-1.3 mm and a volume of approx. 1 mm^3. The surface of the spermatheca is shiny silver. The wall of the spermatheca is hard, translucent and it is covered at the outside with a fine network of windpipes with the role to supply with oxygen the spermatozoa in the seminal liquid found in the spermatheca. In virgin queens, its content is a transparent fluid, like water and in the mated, fecundated queens the liquid inside the spermatheca has a milky, thick aspect, conferred by the penetrated spermatozoa packages.

The spermatic duct (ductus spermaticus) makes the connection from the spermatheca to the oviduct. In its curved portion, the duct is surrounded by developed muscle fascicles which contribute to the propulsion of the sperm in the spermatheca, acting by contraction like a real pump and, probably, play an important role in the release of the sperm. In the spermatic duct there are two ducts of the mucous glands of the spermatheca that open, glands which cover the spermatheca on the outside, and whose secretion plays a nutritive role over the period of keeping the spermatozoa for several years and which act at the same time as a triggering factor of their migration.

The next part of the female genital apparatus is the odd oviduct that we already discussed about above. The odd oviduct opens in the genital chamber. The latter is composed of an external portion (the copulatory sac - bursa copulatrix) that opens at the base of the sting and a portion located in front, corresponding to the vagina.

The vagina represents the terminal portion of the queen's reproductive apparatus. It is composed of an elastic wall, folded, with dips. Due to its elasticity, its shape can easily change depending on the abdomen distension. The vaginal orifice, its connection with the chamber of the sting, is a cross window appearing like a lump, with folds, in the middle part of the bursa copulatrix, being closed in the resting position. The diameter of the vaginal orifice is 0.65-0.68 mm, ranging with age or the moment of the season.

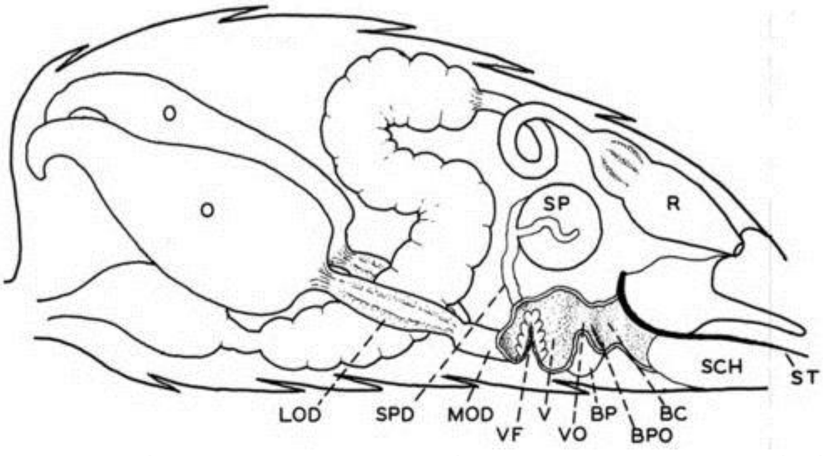

Figure 5. Female reproductive apparatus: BC, bursa copulatrix; BP, right bursal pouch; BPO, opening to bursal pouch; LOD, lateral oviduct; MOD, median oviduct; O, ovary; R, rectum; SCH, the chamber of the sting; SP, spermatheca; SPD, spermal duct; ST, sting; V, vagina; VF, valvefold; VO, vaginal orifice.

3.2. Structure of the colony and sex determination in bee

3.2.1. The social structure of the colony

According to the common understanding, a bee colony is composed of a queen, a variable number of worker bees (and in certain periods of the season, a significantly lower number of drones), brood in various stages of ontogenetical development (their number and stage ranging also based on the season) and honeycombs with stored reserves (Winston 1991; Gould & Gould 1988).

However, this representation is a very superficial description of the colony, which includes only apparent aspects. By its internal structure, the bee colony is a very complex formation, which, despite all the efforts, still fails to be understood even nowadays (Visscher 1980; Ruttner 1980, p.21-23). It is essential to our examinations to understand the multiple relationships that the worker bees share among them and those they share with the brood and the queen (Ruttner 1980, p.21-23).

The same professor Ruttner said that everything began with the fact that a normal bee hive included worker bees of all ages which carried out certain tasks, appropriate to their age. On the other side, the professor said, the organization of these worker bees was not based on some rigid stages in their development. Tasks provided physiologically for a certain age may be temporarily postponed within certain limits or even suppressed, so that it is achieved a high plasticity of the worker bees' tasks, appropriate to the requirements in the hive (see also Pătruică 2005, 2006).

An interesting phenomenon is the way in which the metabolic balance of the colony is maintained by the interaction between individuals. By passing food from bee to bee, a food cycle is formed and, as last resort, a global metabolism of the insect hive where the brood is also included (Page & Erickson 1988; Visscher 1989).

During the first days of life, after it has consumed important amounts of pollen (accumulating proteins), the young bee develops the pharyngeal glands and the body fat. When it is nurse, this worker bee hands over to the larvae the reserves accumulated by the protein by means of royal jelly and, due to this fact, it will be only for a short period a gathering bee, also called flying bee.

Preparing the brood cells, renouncing to food and heat for brood and feeding the queen, the worker bees are the factor in the colony determining the development of events (Ruttner 1980).

The relationships between the worker bees and the queen are very important for the succession of functions inside the bee colony. Mainly the worker bees are those carrying out the vital functions of the hive by various regulatory activities, such as: cleaning of some available honeycomb areas, reduction of the area intended for the brood, indirectly by reducing the amount of royal jelly or directly, by removing some part of the eggs and larvae, which would have been supernumerary to the bearing capacity of the hive at a certain moment. These bees also play an essential role in deciding the amount of male brood which will be increased, but also in increasing the number of young queens. The intensity of the honey and pollen gathering activity is regulated also by the worker bees (Ruttner 1980).

If the worker bees hold directly the decision-making functions in the colony, the queen also has at least an equal influence, not directly but by the means of the worker bees: only in the presence of the queen the worker bees may exert their functions related to the collectivity (Le Conte & Page 2014; Ruttner 1980, p.21-23). The mother bee or queen is the central point of reference, mandatory for the fulfillment of the tasks necessary from the biological point of view. For example, in an orphan colony, the breeding capacity stops, the collecting yield and the defense disposition, as well as the cohesion of the colony. Therefore, the phenomena that occur after the loss of the queen have an almost dramatically nature (Ruttner 1980).

3.2.2. Sex determination in bees

The bees in the *Apis mellifera* species have a reproductive mode and a sex determination according to the haplo-diploid model, i.e. the male are haploids and the females and the worker bees are diploid. In other words, the drones develop from unfecundated eggs, while the queens and the worker bees hatch from fecundated eggs (Adams et al 1977; Mackensen 1951; Beye et al 1996; Hasselmann et al 2001). The visible phenotypic difference between the queen and the worker bee is determined by the specific mode of individual feeding in a given ontogenetical stage (with or without royal jelly). This type of sexuality is not unique in the animal kingdom, approx. 20% of the animal species have a sex determination working by the same model (Gempe & Beye 2009).

3.3. Honey bee reproduction

Based on the degree of involvement of the two sexes in the reproductive act the animal species may be monogamous or polygamous. The monogamous species form couples composed of a male and a female, while in the polygamous species one or both sexes may participate with supernumerary individuals in the reproductive act. Although the classification is much more complicated in reality, the polygamous species may be polyandrous (when a female mates with multiple males) or polygynous (where a male mates with multiple females). The bee, *Apis mellifera* is a polyandrous species (Roberts et al 2015).

The egg of the bee has mainly the general characteristics of an insect egg. The bee egg has in the upper part an opening called micropyl. By this opening the nutritive cells are initially absorbed, and subsequently they form the penetration place of the spermatozoon in view of its fecundation (Chen et al 2017).

The queens become mature and ready for mating after 6 up to 10 days since leaving the queen cells, and the drones much later, i.e. 12 up to 14 days after hatching from the cell.

The young queen is prepared by the worker bees in order to perform the mating flight by intensifying feeding, being also cleaned by licking. This stage in the queen's life may be relatively easy to recognize by the specific position of the queen's abdomen and by the partially open aspect of the chamber of the sting. In the first stages, the queen performs short-term orienting flights, which are then followed by the actual mating flights. The mating flight is carried out in fine weather, in calm and warm conditions, usually in the afternoon, between 13.00 and 16.00 hours, lasting from several minutes to one hour. The queen is mating by multiple couplings, over the course of several flights and each time with multiple drones. The mating flights may take place over the same day or on several successive days. The ideal height of the mating flight is 10-30 m, but the height limits are very large. In some situations it takes place at several meters from the ground and at a variable distance from the hive. This distance can sometimes be several km. During the flight, the queen is followed by numerous drones attracted by the queen's pheromones which are perceived over a 100 m radius.

During the mating, the drone inserts its penian bulb in the queen's chamber of the sting. Inoculation of the sperm takes place at the moment of penis rejection. The penian bulb breaks and remains attached to the rear portion of the queen, called "mating mark". The spermatozoa remain in the vagina, in the median oviduct and in the lateral oviducts, migrating then after 10-18 hours in the spermatheca to be stored. The fecundation process takes place several hours after the mating act and it is produced by the insertion of the spermatozoon in the egg. Simultaneously with this process, a continuous development of the queen's glands takes place, and after a few days the queen begins to lay eggs.

During a flight, the virgin or unprolific and receptive queens mate approximately with 12-14 drones belonging to one or usually several bee colonies (Tarpy & Nielsen 2002; Tarpy et al 2004; Amiri et al 2016, 2017), but they keep less than 10% of the seminal volume (Koeniger 1986) and approx. 3% of the live spermatozoa received by copulation (4-7 million spermatozoa, Harbo 1986; Wilde 1994; Collins 2000; Baer 2005; Cobey 2007). These values correspond to a volume of 8-12 μL of spermatic material (Mackensen 1964; Woyke 1989; Cobey 2007), which is gradually and economically used by the queen (Baer et al 2016) for the daily fecundation of a number of 1500-2000 worker bee eggs (Winston 1987).

3.4. Obtaining the queens

Obtaining the queens is one of the most important aspects of the reproduction, be it developed in semi-natural conditions, or by artificial insemination; therefore, we will pay a special attention to the detailing of this subchapter. Out of the selection criteria we will retain, in descending order: the honey production, the fertility of the queens for at least one season, the resistance to diseases, the docility, the capacity to spend the winter in good conditions, the swarming tendency.

3.4.1. Obtaining and utilization of queens from natural swarming

The evolutionary biologists say that bees exist for more than 50 million years (Cridland et al 2017; Corona & Robinson 2006). They live and breed very well in the absence of humans. The reproduction of the bee colony is made naturally by swarming, a rustic behavior which ensures the perpetuation and spreading of these insects (Raffiudin & Crozier 2007).

During spring, usually in May, when conditions become favorable, a part of the population follows the queen in order to create a new hive. Most of the times, this swarm remains attached to a branch for a few hours, until the scout bees find a new shelter somewhere. The remaining, orphan worker bees will grow a new queen, starting from the young larvae. Sometimes, they build several queen cells, not just one. Some virgin queens resulting from these queen cells will leave the hive, accompanied by a few bees to compose swarms called secondary swarms (Fert 2011).

The colonies produce queen cells in three situations:

1) in case they prepare to swarm;
2) when the colony is left orphan and
3) when it quietly changes its queen.

The use of queens from the swarming queen cells is not recommended. Their repeated use may lead to the consolidation and amplification of the swarming behavior. Thus, the beekeeper is faced with the situation that in the swarming period he cannot stop the triggering of this phenomena, as the losses are high, the hives grow weaker and the yield decreases significantly.

For a long time, it was believed that the queens coming from swarming are more valuable than those achieved artificially. However, subsequent research has proven that by carefully applied and controlled breeding methods there are obtained queens at least of the same value as those in the swarming and natural division (Dobrotă 2011).

In modern beekeeping, the natural swarm is not a desired phenomenon (Laidlaw & Page 1997), or at least it should not be one. The mentality of some beekeepers who wait for the natural swarming as being the only breeding or hive repopulation manner should be reviewed and replaced by the scientific and technical principles of artificial reproduction. These have a multitude of advantages, as they are easy to perform, with the controlled intervention of the beekeeper. Thus, they will have queens that will transmit the swarming characteristics, which is desirable if we intend to have hives with good hereditary features in order to achieve a good yield of honey and pollen (Tudor 2011).

As previously mentioned, when the colony is swarming, the worker bees build numerous swarming queen cells, mainly from the sides and the bottom of the honeycombs. The queen cells built by the bees during the swarming fever period may be used to achieve young queens. How is it done? As the queen cells develop and become mature, they are cut together with a piece of honeycomb one or two days before the queen hatches. The mature queen cells, where the queens are close to hatching, may be recognized by their color. They have a darker color, and the cap is light colored, with a fibrous and partially eroded aspect. The swarming queen cell is detached from the old honeycomb by means of a very sharp and heated knife, together with a 1-1.5 cm wide piece of honeycomb and it is grafted with a well built, darker honeycomb, which has already produced a few brood generations, preferably with a capped brood and a honey crown at the upper part, in an orphan hive or a recently built artificial swarm.

In order to achieve queen cells from a certain hive one can use the method of inserting swarming fevers of the hive one intends to collect the queen cells from. In this sense, the respective hive is ensured the optimum development conditions early in the spring, for the brood and young specimens' growth. This means enough food, narrow nest on as few as possible honeycombs to keep an adequate thermal regime, one year queen, and the protection of the hive against the cold air currents. During the second half of the spring (usually in May), when the hive is at its development climax, with many young bees, one should stop inserting new frames used for nest expansion and keep the nest cramped. In addition, one may strengthen with capped, ready-to-hatch brood. The surplus of young bees, the lack of space to store the honey and the lack of empty cells necessary for the egg laying will force the colony to go into swarming fever and build a large number of queen cells. These queen cells may then be used by the beekeeper according to the needs, by detaching them according to the aspects presented above or the queen cells may be taken out with the entire frame as their cutting is no longer necessary. For the formation of an artificial swarm only one or maximum two queen cells are left. These are well developed by the bees, while the rest of the queen cells must be destroyed to avoid multiple swarming.

From the swarming queen cells corpulent queens may be achieved, with a very well developed abdomen and very prolific, but they are (or at least they can very probably be) swarming as behavior, similar to the hives they come from. Their characters have a hereditary foundation. Some of the specialists say that for this reason using these queens for reproduction is not recommended, as the pollen and honey yield is often compromised by the frequent going of the colonies with such swarming queens in the swarming fever.

3.4.2. Obtaining queens from the queen cells taken from the orphan colonies

When the time for swarming is near or at the beginning of the harvest, the removal of the queen from a hive enables the achievement of several queen cells, sometimes more than twenty. In approximatively 10 days after becoming orphan, all these queen cells, except one, carefully detach from the frames, so as not to get damaged. The remaining queen cell, usually the most developed of them, ensures the existence of a queen in the raising hive and the detached ones, already capped, are distributed into new swarms or nuclei formed in the hives that became orphan 2-3 days before. The success of this method depends very much on the ethology of the bees in the respective hive. On the other hand, in this case, the selection and multiplication possibilities are rather limited (Fert 2011). The queen that will come out of this queen cell will produce satisfying results for approximately one year and then it will have to be changed.

3.4.3. Obtaining the queens from queen cells produced for calm change

When the queen is old, exhausted or when it is mutilated as a result of an accident or as a result of the beekeeper's intervention, the worker bees build maximum 2-3 large queen cells, usually on the central part of the honeycombs that will produce high-quality queens. Many beekeepers consider that these queens, coming from the queen cells for quiet change, would be the best.

The beekeepers may obtain queen cells for quiet change in three stages:

I. - Settlement of the most productive hive/hives, with particular biological qualities.

II. - the amputation by means of some scissors of the front wings or a front leg of the old queen that needs to be replaced but without causing any trauma to any vital anatomic parts (such as the head, the thorax, the abdomen), so that the queen can continue to lay eggs under normal circumstances. Due to this apparent infirmity (the queen does not use its wings for egg laying) the bees in that particular colony decide to replace it by quiet change. Thus, they build 2-3 queen cells in the middle of the honeycombs where the queen will lay eggs forced by the worker bees. Then the worker bees will abundantly feed the larvae in these queen cells with royal jelly so that future mothers benefit from the best development conditions from the beginning of the growth.

III. - After 10-12 days from the moment of amputation, the presence, number and position of the queen cells are checked. When the queen cells reach maturity (around

12-14 days old), they notch off and project a number of swarms equal to the number of queen cells or queen cells frames there are. Two more capped brood honeycombs per each newly formed swarm add to these. These are ready to hatch and produce honey and they are from the same hive in which the queen cells have developed, as well as a frame with artificial honeycomb for building.

If it is necessary to further produce queen cells raised under quiet change circumstances then we leave the disabled queen in its hive. The nest is supplemented with 2 or 3 honeycombs built from the back-up and 2 or 3 honeycombs with capped brood from other hives. Following the same cycle of 12 to 14 days, the procedure is repeated: in the nest there are 2-3 mature queen cells, close to hatching, which will be used in view of multiplication. The repetition of this cycle is carried out until the complete number of queens in the apiary is obtained. During the last queen cells harvesting cycle, when no other queen cells are wanted, one frame with one queen cell is left in that hive. The young queen that hatches will substitute its old and disabled mother.

The swarm formed this way, with queens raised by quiet change, is stimulated every two days with 200-400 g inverted sugar syrup until the queen starts to lay the first eggs. After the hives with young queens have achieved the autonomy necessary to a developing hive, one proceeds to the merging of the hives whose queens should be replaced with these swarms with young queens.

3.4.4. Use of Jenter box or Nicot box

The Jenter and Nicot boxes are the best known and are very similar. The Jenter box is a device composed of several plastic elements. This enables the breeding of the queens in artificial queen cells, made of plastic. The larvae transplants are no longer necessary, as the queen lays its eggs directly into the block mounted queen cells, in the plastic strip in the form of honeycomb. It is a pretty good procedure as the quality of the queens is high, these being obtained early and the eggs are laid directly in the queen cells by the queen. However, it is a time consuming method, it needs more steps and it is less preferred by the industrial queen providers.

3.4.5. Larvae transplants

This method is used due to its efficiency, rapidity and good results achieved after its application in the apiaries of the large beekeepers and by most of the professional queen breeders and it has two options: the moving of the queens into queen cells without larvae food (royal jelly), which is called dry or simple transplant and the movement of larvae into queen cells where there is royal jelly, which is called wet or double transplant. Larvae transplant is basically the transfer of one worker larvae in an artificial queen cell.

For the implementation of the larvae transplant activity in order to obtain queens, there are a few techniques in the published documentation. The most familiar are: Doolittle, Bentley, Miller and Alley.

Regardless of the technique or the method used in the larvae transplant activity, in order to achieve quality queens it is necessary to make a rigorous selection, both of the source hive and of the breeder bees. The hives should be very well populated, the pollen available for the nurse bees should be in high amounts. There should be performed a stimulation with syrup after each handling, the larvae that will be transplanted should be as young as possible (1-2 days old), and the time interval from the moment of transplant to the insertion into the starter of the artificial queen cells (wax or plastic cell cups) should be as short as possible.

3.5. Breeding the drones and ensuring reproduction conditions

In order to obtain a high quality biological material the mating apiaries need to have a sufficient number of high quality males, selected from hives known to be productive and of known origin (Căuia 2005; Pătruică & Bura 2017).

At the same time, the breeding of drones has several objectives: birth of selected and high quality males, obtaining more drones than necessary, prolongation of the fecundation period, maintenance of a maximum genetic variability (Fert 2011).

In order to achieve these challenges, some actions are needed, such as: providing the hives that produce drones (also called father-hives) with selected queens, carrying valuable characters, but also planning the male breeding activities depending on the season moment.

In order for the drones to be able to reproduce when the first queens obtained in the apiary perform their mating flight, it is necessary that the beginning of the male breeding works is 2-3 weeks in advance as compared to the female breeding works. This period overlaps the blossom time of the fruit trees, and the determination of this temporal offset is possible considering: queens' metamorphosis cycle, 16 days from the egg stage to hatching and the drones' cycle which is 24 days, the sexual maturing (7-10 days for the queen and 10-14 days for the drone). Beside these periods, the breeder should also consider the time necessary for the preparation of the egg laying preparedness by the worker bees, which is 2-3 days.

In order to breed males, the nests of the father-hives should be reduced as much as possible (by this measure the queen is forced to lay as many drone eggs as possible and the nest is better protected in terms of heating, as the bees cover entirely the honeycombs in the nest), the colony is stimulated with mixed additional food, i.e. inverted sugar syrup and protein cake. After that, an artificial honeycomb with drone cells is inserted between two honeycombs with young individuals, a honeycomb built with drone cells mainly or a frame for building (empty, only the wooden frame, as the bees build in it the honeycombs with cells for the future drones).

After approximately one week from the insertion of the honeybees, the beekeeper should check the nests of those hives and he needs to see whether the queen has laid eggs in these honeycombs. The honeycombs where there were no eggs laid are taken away and are replaced by honeycombs with eggs, taken from other bee hives. In this way, both the drone larvae breeding in the hives where the egg honeycombs were inserted and the egg population of all empty honeycombs with drone cells are ensured. In a father-hive there can be mentioned two-three honeycombs with drone cells.

The queen can be placed also in an isolation cage to lay the drone eggs in the frame placed in the nest. The hive is strengthen by placing a frame with capped brood, in a stage close to hatching, taken from another stronger and more productive hive.

In order to limit the breeding of drones in the bee hives and this way to favor the mating of the queens only with those drones produced in the father-hives, the beekeeper may employ various methods, such as: using only high quality honeycombs with mainly worker bee cells, the cutting out of the drone cells honeycombs (from the building frames or from the edges of the built frames in the hive), uncapping of the drone brood cells, use of Hanneman bars for the hive entrance, use of special traps for drones or the pollen collectors.

Similar to other insects (Parker 1970), in the bee communities there is a competition at the gametes level, by internal and behavioral physiological mechanisms, thus favoring the seminal material or the gametes of certain males considered by nature as being the most viable or as generating viable offsprings. The phenomenon has also been found in other animals (Requena & Alonzo 2017; Simmons 2014), such as fish (Mag-Mureșan & Bud 2004), birds (Albrecht 2017), mammals (Ginsberg & Huck 1989; Stockley 2004; Hosken 1997). This trend of the queen to select its gametes considered more viable or more adapted makes the beekeeper's efforts more difficult in selecting the adequate genitors and in producing high performance, productive, desired animals.

In order to ensure the optimum mating conditions of a number of 50-70 queens it is necessary to breed drones in a single hive. By special breeding of the drones one cannot ensure a 100% controlled mating, but one can achieve a rather high percentage of mating with the selected drones, valuable from the hereditary point of view, both in terms of behavior and morphoproductivity. A large part of the offsprings will be the descendants of other increased fitness drones, but not with the best genetic and morphoproductive qualities. On the other hand, absolutely promiscuously, the queen can mate also with some drones that are inferior from many points of view. The safety of queens' mating exclusively with desired drones, from selected hives, is achieved exclusively by using artificial insemination of the queens (Laidlaw 1944).

Approximately three weeks before the date when the breeding of the last queen batch is planned, the production of drones in the specially selected hives, may be stopped. Bees reproduction carries out as presented in subchapter 3.3.

3.6. Artificial insemination: common techniques and their utility

Artificial insemination (according to other researchers - instrumental insemination) was successfully employed for the first time by Laidlaw (1944), Woyke (1962) and significantly improved by Page et al (2002) and Cobey et al (2013) for *Apis mellifera*. The method was adapted and successfully employed by Phiancharoen et al (2011) for the artificial insemination of the species *Apis florea*, and Woyke (1973, 1975) adapted it and employed it for *Apis cerana*.

Also, many interspecific hybridization attempts by artificial insemination were unsuccessfully made (Koeniger et al 1998; Phiancharoen et al 2004; Ruttner & Maul 1983; Woyke 1993; Woyke et al. 2001).

Artificial insemination has occurred as a more "sophisticated" alternative to systematically control the queens' mating by monitoring the mating process used by considering the queens and drones selection from the colonies with superior, elite features (Laidlaw 1944).

This method is used mainly in areas unfavorable for natural safe mating, it generates very good results and it provides substantial advantages in improving the reserves, but it also maintains pure features (Cobey et al 2013).

In practice there is a technique that uses specialized equipment for anesthesia, immobilization and insertion of the sperm in the vaginal tract (oviduct) of the queen with seminal material collected from the mature drones by manual eversion of the endophallus.

An important aspect that needs to be mentioned is that, after the artificial insemination, the queen has the same performance as in the case of natural mating (Cobey et al 2013).

In order to conduct this activity it is necessary to meet several mandatory requirements:

- in the laboratory (the space where the artificial insemination take place, under aseptic conditions and controlled climatization), temperature and humidity should imitate the natural mating conditions;
- the mating nuclei where the unprolific queens will be inserted should be populated with bees; the nuclei should be provided with Hanneman bars hive entrance so that the queens should come from "mother" bee types of hives which meet the previously established selection conditions;
- the "father" bee hives should be available, i.e. those hives where those drones the sperm will be collected from are kept.

Also, artificial insemination implies the use of specific equipment and tools such as: the macroscope, the carbon dioxide bottle, cold light lamp, the tweezers, the special hooks (dorsal and ventral), the insemination device, the insemination syringe, the tubes for queen fixation, the queens' keeping cages, the drones' exploitation cages, but also other specific accessories (Laidlaw 1944; Cobey et al 2013).

3.6.1. Instruments and accessories necessary for artificial insemination of queens

Further on there will be presented the instruments necessary for beekeepers who wish to benefit from the best hereditary features of bees by means of artificial insemination technique.

3.6.1.1. The macroscope

It is an optical device similar to the microscope, but with a considerably lower zooming capacity, which is used in order to have a better visibility during the insemination. It is recommended to use the zooming capacity system ranging between 10X and 20X.

The macroscope can be monocular or binocular. The binocular macroscope is recommended as it enables examination with both eyes, and the comfort is enhanced when both eyes remain open. Thus, the practitioner can work easier with the insemination device.

Optionally, the macroscope can be endowed with a video camera that can send the signal to the monitor. The entire insemination procedure can be watched on this monitor or recorded on electronic support.

3.6.1.2. The carbon dioxide bottle

The carbon dioxide bottle is necessary for anesthetization of the queens subjected to the artificial insemination intervention.

Carbon dioxide is used because it has a numbing effect on the queens (Mackensen 2014), so that they are docile and quiet during the intervention and they allow the insertion of the insemination syringe needle in their vagina.

The pressure of carbon dioxide which goes into the tube where the queen id fixed should be constant, and the CO_2 amount should be correctly dosed. An amount of carbon dioxide too large administered all at once may have irreversible harmful effects on the queen. That is why the carbon dioxide bottles are provided with a pressure regulator.

Even in the optimum usage conditions, the effects of anesthesia and handling of the queen cause stress, they are felt by the queen and the cascade of adverse reactions may be transmitted to the worker bees (Richard et al 2011). Under the effect of anesthesia, the egg laying procedure is carried out in a hurry and sometimes the queens put to sleep with CO2 lay their eggs even if they are non-fertilized (Kaftalongu & Peng 1982). The anesthetization of the queens with carbon dioxide is the safest method to induce egg laying (Mackensen 1947; Ebadi & Garry 1980; Janoušek 1987; Konopacka 1991; Gerula et al 2011, 2012), and this is desirable after the beekeeper's insemination work.

The amount of carbon dioxide coming out of the tube may be monitored in a water bowl; the formation frequency of the water bubbles is an empiric indicator (but of great help) of the ejected CO_2 flow.

3.6.1.3. The cold light lamp

It is a lamp that emits light rays, except infrared rays (heat). The light is cold as all the thermal radiation has infiltrated inside this lamp. In the artificial insemination it is important to use this type of light radiation because the cold light beams emitted by the lamp give a better visibility of the queen's abdomen and there are thus avoided the heating, the drying of the membrane and the irritation of queen due to the use of full-spectrum light. Therefore, the queen is not excessively heated during the intervention.

3.6.1.4. The tweezers

The tweezers is a tool used for holding the queen's sting. By pulling the sting towards the lateral margins of the insect's abdomen, the vagina is exposed, with the sport for inserting the insemination syringe. The abdomen may then be maintained open, at its distal end, by using the hooks from the insemination device. The tweezers is also used for seizing, handling and applying the number plates on the thorax of the inseminated queens.

3.6.1.5. The insemination device

The main tool used in artificial insemination of the queens is composed of several components, which differ from one device to another. On the market there can be found several types of queen insemination devices, but they are all based on the same operating principles, and the use of a certain type depends on the technicians' preference. The insemination device is composed of a queen immobilization system which includes one or two hooks, depending on the device, a syringe containing the seminal liquid collected from the males and a syringe mechanical guidance system in the desired position.

3.6.1.6. The tube for queen fixation

In order to be inseminated, the queen is fixed in a transparent tube made of plastic, with one of its ends of a conic shape and narrower (from 6.6 to 4.8 mm internal diameter). In order to insert the queen in this holder one uses a tube of the same diameter, but closed at one end.

3.6.1.7. The insemination syringe

The insemination syringe is the tool used for collecting the drone sperm and it is also used for inseminating the queen.

There are several variants on the market. We will mention only two: Schley and Harbo (Figures 6 & 7). They are basically built of a tube (metallic body) where a transparent acrylic tube is placed. The metallic tube has an inside thread for both ends. In one end a metallic piston is threaded and in the other end the sperm collecting needle is fitted by means of a membrane and a coupling.

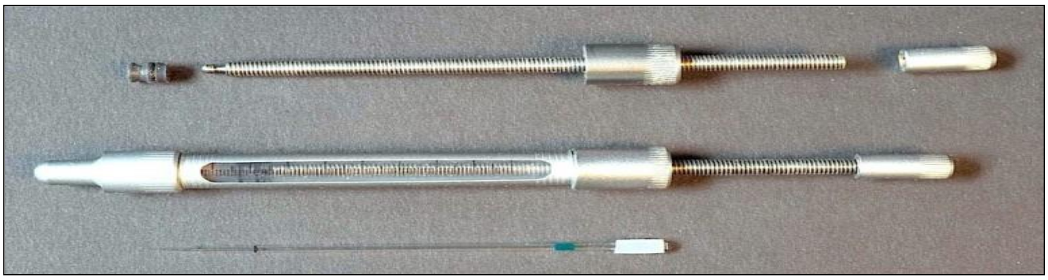

Figure 6. Schley syringe (source: http://www.besamungsgeraet.de/__en/insemination-instruments/).

Figure 7. High-capacity Harbo syringe (original).

3.6.1.8. The ventral hook, the dorsal hook and the bumper

These are special tools used for gripping and removing the last sternite of the queen, the queen's sting apparatus and for removing (pushing) the soft tissues that cover the vaginal orifice (Cobey et al 2013).

3.6.1.9. Other accessories used for insemination

Other accessories are: the salt solution when pumping the semen through the syringe, white vaseline for sealing the sperm tubes, sterile wet napkins, Sharp scissors, cages for queens and drones, distiled water, holder, 96% concentration alcohol.

3.6.2. The procedure for collecting sperm from drone

The drones differ very much from one another in terms of the easiness they ejaculate, but also by the sperm volume that can be collected from one individual, especially when consanguine genetic material is used. Some drones do not have sperm; in others the turning of the endophallus and the ejaculation do not occur naturally when artificially stimulated; while in others the turning is so violent that the sperm is projected and lost or the endophallus explodes (Mackensen & Ruttner 1976).

The correct immobilization of the drone when collecting the sperm is extremely important. In order to cause eversion and correct ejaculation, the head and thorax of the drone are grabbed between the thumb and the index finger of the left hand, holding the drone with the ventral part up. Then, while the head and the front part of the thorax are gripped in the left hand, the dorsal part of the abdomen is slightly and repeatedly squeezed between the thumb and the index finger of the right hand. This action usually causes the contraction of the abdominal muscles and a more or less complete eversion of the penis and ejaculation. If the penis is only partially turned and the sperm does not occur, the abdomen is progressively squeezed from the dorso-front part towards the ventro-posterior part in order to continue the forced turning of the penis until the sperm is eliminated. The sperm is rarely obtained without abdominal contraction, but when the abdomen is contracting without the partial turning of the penis, this can be fully turned by pushing it, thus achieving a larger amount of sperm (Mackensen & Ruttner 1976).

The amount of sperm and mucus eliminated are variable in the drones. As the penis is turned over, the cream-colored sperm appears first, followed by the white, thicker mucus. Sometimes only sperm is released, but usually, after sperm, a small amount of mucus comes out, both spreading on the upper part of the penis, in varying proportions. The spermatozoa movements cause the sperm to spread in a thin layer over the mucus layer, which often renders its collection with the syringe very difficult. For this reason, it is important that any delay is avoided when collecting the sperm (Mackensen & Ruttner 1976).

The drone that has just ejaculated is brought to the top of the syringe with the left hand. The piston is withdrawn to create an air bubble between the sperm and the salt solution and to estimate the collected amount of sperm. The sperm surface is then brought into contact with the tip of the syringe, under an angle of approximately 45°. If the drone is slowly withdrawn from the tip of the syringe, without interrupting their contact, the sperm will continue to adhere to it and will flow as the piston is withdrawn (unfastened). This procedure helps the operator avoid the sampling of the mucus – as it is viscous it does not flow. The mucus is too consistent to go into the tip of the syringe and would stop the passing of the sperm. If this accident occurs, the piston must be unfastened (advanced) until the lumen is clean; then the sperm collection operation is resumed. The drone sperm is collected until the syringe is filled to the desired level. The amount of sperm that can be collected from one drone is approx. 0.8-1 µl. The beginner operator must sample the sperm very slowly; with experience, he will be able to work faster (Mackensen & Ruttner 1976).

When the insemination device has been changed so that the fixing of the queen happens very fast, many times the insemination works conversely, i.e. The sampling of the sperm in the syringe – fixing and anesthetization of the queen – insemination with seminal material. If mature drones are available, the time needed for the procedure is as follows: filling of the syringe - 8 minutes, insemination - 2 minutes. If all is well prepared, approx. 15 minutes are calculated on average for one insemination, including changing the queens and getting the drones (Mackensen & Ruttner 1976).

3.6.3. The artificial insemination procedure

The number of descendants that a queen produces during its lifetime depends directly on the amount of seminal material collected during mating (or, in case of artificial insemination, on the insemination volume) and the number of viable spermatic cells contained in the seminal material (i.e. the sperm viability).

Various specialists on several occasions have proven that the insemination volume of the female changes the mandibular pheromone profiles of the queen (Richard et al 2007), the chemical profile of the Dufur gland (Richard et al 2011), the vitellogenin level (Kocher et al 2009), the genic expression in the queen's and worker bees' brains (Kocher et al 2008, 2009, 2010; Niño et al 2013), as well as the behavior and physiology of the worker bees at individual level (Richard et al 2007; Kocher et al 2009; Niño et al 2012).

To determine whether the insemination volume has significant effects at colony level, Payne & Rangel (2018) have compared the evolution of the hives with artificially inseminated queens which have been inseminated a reduced volume of spermatic material (1.5 μL) with other queens artificially inseminated with a larger volume of spermatic material (9.0 μL) collected from May until October, between 2013 and 2015. The researchers did not find any significant different effect caused by the variation of the insemination volume on the yield, harvest, number of worker bees or on the survival of the colonies, at least during the first year of a bee hive’s life (Payne & Rangel 2018). However, the studies showing the effects of the insemination volume and of the diversity of its origin on the colony individuals (Richard et al 2007, 2011) should not be neglected.

The queens are inseminated between 6 and 12 days after their hatching from queen cells, when they become mature. According to some researchers, it is ideally to do this on the eighth day (Ruttner 1976). The procedure is carried out in several stages. According to the state of the art presented in the published literature (Cobey et al 2013), with minor changes from one practitioner to another, these stages are:

1) Two carbon dioxide (CO_2) treatments. The first treatment implies an exposure of 5 minutes the most and it is achieved one or two days before the artificial insemination procedure. The second treatment is applied during the procedure itself. It is important to mention here that, if this second anesthetization is performed correctly, it stimulates the oviduction of the queen.

2) The syringe and the queen holder are aligned on the tool holder at an angle of 30°, 45° or 60° (depending on the tool that is used) to facilitate the penetration of the syringe needle into the vaginal orifice.

3) Then, the queen is inserted inside the retention tube of the insemination device for immobilization and fixation, head down, towards the carbon dioxide source. A continuous, slow flow of carbon dioxide is administered to put the queen to sleep.

4) The visible folds of the abdomen are separated, more precisely the sternite and the tergite, and the chamber of the sting to expose the vaginal orifice, using a pair of hooks or tweezers.

5) The syringe needle tip is placed at the rear part, above the "V spot", defining the vaginal orifice. The tip of the needle is inserted in the vaginal orifice, at a depth ranging between 0.5 and 1.0 mm, slightly before the tip of the "V spot".

6) The tip is further inserted in depth, for another 0.5 to 1.0 mm, while making a slight zig-zag movement to bypass the entrance valve in the vaginal orifice.

7) A precise amount of seminal material is inserted directly into the median oviduct. The standard injected dose is 8-12 μl for each queen. When being administered the spermatic material, the queen is inserted directly into the colony of its mating nucleus to facilitate the migration of the sperm and/or to be able to administer two doses of 6 μl of spermatic material in a 48 hours interval. Otherwise, the maximum volume of spermatic material cannot fit the median and collateral oviducts.

8) At the end of the insemination, the tip of the syringe collects a small air space with a small drop of salt solution (approximately 0.5 μl) for the next insemination, only to prevent the drying of the sperm.

Discussion. The published literature mentions that in order to reduce the risk of sperm and queen contamination with pathogenic agents it is recommended that the drones eliminate the fecal matters before collecting sperm from them (Ruttner 1976). However, it is not specified how this is performed. Moreover, the literature does not mention whether the queens are allowed to fly before the insemination.

Conclusions. In Romania, due to the pedo-climatic and environmental conditions, the selection, improvement and maintenance in pure state of the selected genetic material may be conducted in a 100% controlled manner by instrumental insemination.

The published literature mentions that in order to reduce the risk of sperm and queen contamination with pathogenic agents it is recommended that the drones eliminate the fecal matters before collecting sperm from them. However, it is not specified how this is performed. Moreover, the literature does not mention whether the queens are allowed to fly before the insemination.

In a future study we will investigate the importance of drones and queens dejection before performing the insemination procedure, as we presume that there is a real contamination risk of the seminal material, of the tools and implicitly of the queens with fecal matters, which can compromise the result of instrumental insemination.

References

Adams J., Rothman E. D., Kerr W. E., Paulino Z. L., 1977 Estimation of the number of sex alleles and queen matings from diploid male frequencies in a population of *Apis mellifera*. Genetics 86(3):583-596.

Aioanei F., Stavrescu-Bedivan M.-M., 2011 Zoologia nevertebratelor. Manual Universitar. Editura Bioflux, Cluj-Napoca.

Albrecht T., 2017 Avian sperm competition. In: Encyclopedia of evolutionary psychological science, Springer International Publishing, pp.1-4.

Amiri E., Meixner M. D., Kryger P., 2016 Deformed wing virus can be transmitted during natural mating in honey bees and infect the queens. Scientific Reports, 6, 33065.

Amiri E., Strand M. K., Rueppell O., Tarpy D. R., 2017 Queen quality and the impact of honey bee diseases on queen health: Potential for nteractions between two major threats to colony health. Insects 8, 48.

Baer B., 2005 Sexual selection in *Apis* bees. Apidologie 36(2):187-200.

Baer B., Collins J., Maalaps K., den Boer S. P., 2016 Sperm use economy of honeybee (*Apis mellifera*) queens. Ecology and Evolution 6(9):2877-2885.

Ball B. V., Allen M. F., 1988 The prevalence of pathogens in honey bee (*Apis mellifera*) colonies infested with the parasitic mite *Varroa jacobsoni*. Annals of Applied Biology 113(2):237-244.

Batin P., Roman V., 2016 Evolutionary species concept. Presentation. Sesiunea Anuală de Comunicări Ştiinţifice Studenţeşti, USAMV Cluj, Cluj-Napoca.

Beekman M., Ratnieks F. L. W., 2000 Long-range foraging by the honey-bee, *Apis mellifera* L. Functional Ecology 14(4):490-496.

Beye M., Moritz R. F. A., Crozier R. H., Crozier Y. C., 1996 Mapping the sex locus of the honeybee (*Apis mellifera*). Naturwissenschaften 83(9):424-426.

Bucată P., 2001a Pledoarie pentru creșterea albinelor. Editura Alex-Alex, București.

Bucată P., 2001b Polenul în hrana albinelor (The pollen in bee feed). ACA, Bucureşti, pp. 47-51.

Bura M., Pătruică S., Grozea I., 2003 Contribuţia polenizării albinelor la sporirea calitativă şi cantitativă a producţiei alimentare. Buletinul AGIR, anul, 8, 79-82.

Căuia E., 2005 Cercetări privind îmbunătăţirea tehnologiei însămânţărilor artificiale la albine. Teză de doctorat. Facultatea de Zootehnie. Universitatea de Ştiinţe Agronomice şi Medicină Veterinară, Bucureşti.

Celli G., Maccagnani B., 2003 Honey bees as bioindicators of environmental pollution. Bulletin of Insectology 56(1):137-139.

Chen L., Xu X., Zhu X., Zhou S., Wang Q., Hao Z., Zhou B., 2017 Effects of low temperature stress on hatching rate and developmental duration of fertilized eggs of Italian honeybees (*Apis mellifera ligustica*). Journal of Environmental Entomology, 39(1):111-117.

Cobey S. W., 2007 Comparison studies of instrumentally inseminated and naturally mated honey bee queens and factors affecting their performance. Apidologie 38:390–410.

Cobey S. W., Tarpy D. R., Woyke J., 2013 Standard methods for instrumental insemination of *Apis mellifera* queens. In: Coloss Bee Bool. Vol.I., Standard Methods for *Apis mellifera* Research. International Bee Research Association, Department of Entomology, Washington State University, USA.

Collins A. M., 2000 Relationship between semen quality and performance of instrumentally inseminated honey bee queens. Apidologie 31:421–429.

Cornoiu I., 2012 Albine şi oameni. Editura Risoprint, Cluj-Napoca.

Corona M., Robinson G. E., 2006 Genes of the antioxidant system of the honey bee: annotation and phylogeny. Insect Molecular Biology 15(5):687-701.

Costea C., Stroia A., 2011 Învaţă de toate. Editura ASE, Bucureşti.

Cridge A. G., Lovegrove M. R., Skelly J. G., Taylor S. E., Petersen G. E. L., Cameron R. C., Dearden P. K., 2017 The honeybee as a model insect for developmental genetics. Genesis 55(5), e23019.

Cridland J. M., Tsutsui N. D., Ramírez S. R., 2017 The Complex Demographic History and Evolutionary Origin of the Western Honey Bee, Apis Mellifera. Genome Biology and Evolution 9(2):457-472.

Crozier R. H., Crozier Y. C., 1993 The mitochondrial genome of the honeybee *Apis mellifera*: complete sequence and genome organization. Genetics 133(1):97-117.

Dade H. A., 1994 Anatomy and dissection of the honeybee. IBRA.

Dezmirean G. I., Mărghitaș L. A., Bobiș O., Dezmirean D. S., Bonta V., Erler S. 2012 Botanical origin causes changes in nutritional profile and antioxidant activity of fermented products obtained from honey. Journal of Agricultural and Food Chemistry 60(32):8028-8035.

Dobrotă V., 2011 Creșterea mătcilor. Editura Salgo, Sibiu.

Ebadi R., Garry N. E., 1980 Factors affecting survival, migration of spermatozoa and onset of oviposition in instrumentally inseminated queen honeybees. Journal of Apicultural Research 19(2):96-104.

Estoup A., Garnery L., Solignac M., Cornuet J. M., 1995 Microsatellite variation in honey bee (*Apis mellifera* L.) populations: hierarchical genetic structure and test of the infinite allele and stepwise mutation models. Genetics, 140(2):679-695.

Estoup A., Solignac M., Harry M., Cornuet J. M., 1993 Characterization of (GT) n and (CT) n microsatellites in two insect species: *Apis mellifera* and *Bombus terrestris*. Nucleic Acids Research 21(6):1427-1431.

Evans J. D., Aronstein K., Chen Y. P., Hetru C., Imler J. L., Jiang H., Hultmark D., 2006 Immune pathways and defence mechanisms in honey bees *Apis mellifera*. Insect Molecular Biology 15(5):645-656.

Fert G., 2011 Creșterea reginelor. Editura Lumea Apicolă.

Garnery L., Cornuet J. M., Solignac M., 1992 Evolutionary history of the honey bee *Apis mellifera* inferred from mitochondrial DNA analysis. Molecular Ecology 1(3):145-154.

Gempe T., Beye M., 2009 Sex determination in honeybees. Nature Education 2(2):1.

Gerula D., Bieńkowska M., Panasiuk B., 2011 Instrumental insemination of honey bee queens during flight activity predisposition period 1. Onset of oviposition. Journal of Apicultural Research 55(2):53-66.

Gerula D., Panasiuk B., Węgrzynowicz P., Bieńkowska M., 2012 Instrumental insemination of honey bee queens during flight activity predisposition period 2. Number of spermatozoa in spermatheca. Journal of Apicultural Science, 56(1):159-167.

Ginsberg J. R., Huck U. W., 1989 Sperm competition in mammals. Trends in Ecology & Evolution 4(3):74-79.

Goblirsch M., 2017 Using Honey Bee Cell Lines to Improve Honey Bee Health. In: Beekeeping–From Science to Practice. Springer, Cham., pp.91-108.

Gould J. L., Gould C. G., 1988 The honey bee. Scientific American Library.

Harbo J. R., 1986 Propagation and instrumental insemination. In: Bee breeding and genetics. Rinderer T. E. (ed), pp. 361–389, Academic Press, Inc., Orlando, FL.

Hasselmann M., Fondrk M. K., Page R. E., Beye M., 2001 Fine scale mapping in the sex locus region of the honey bee (*Apis mellifera*). Insect Molecular Biology 10(6):605-608.

Hosken D. J., 1997 Sperm competition in bats. Proceedings of the Royal Society of London B: Biological Sciences 264(1380):385-392.

Janoušek J., 1987 Effect of carbon dioxide on initiation of oviposition of inseminated queens. Vedecke Prace, V.U.V. v Dole, 9:57-64.

Kaftanoglu O., Peng V. S., 1982 Effects of insemination on the initiation of oviposition in the queen honeybee. Journal of Apicultural Research 21(1):3-6.

Kocher S., Richard F. J., Tarpy D. R., Grozinger C. M., 2008 Genomic analysis of post-mating changes in the honey bee queen (*Apis mellifera*). BMC Genomics 9, 232.

Kocher S., Richard F. J., Tarpy D. R., Grozinger C. M., 2009 Queen reproductive state modulates pheromone production and queen-worker interactions in honey bees. Behavioral Ecology 20:1007-1014.

Kocher S. D., Ayroles J. F., Stone E. A., Grozinger C. M., 2010 Individual variation in pheromone response correlates with reproductive traits and brain gene expression in worker honey bees. PLoS ONE. https://doi.org/10.1371/journal.pone.0009116

Koeniger G., 1986 Reproduction andmating behavior. In: Bee breeding and genetics. Rinderer T. E. (ed), pp. 235-252, Academic Press, Inc., Orlando, FL.

Koeniger G., Koeniger N., Tingek S., Kelitu A., 1998 A preliminary report on hybrids between *Apis cerana* Fabricius, 1793 and *Apis koschevnikovi* v. Buttel- Reepen, 1906 produced by instrumental insemination. Apidologie 29:462–463.

Koeniger N., Koeniger G., Tingek S., 2010 Honey bees of Borneo: exploring the centre of *Apis* diversity. Borneo: Natural History Publications.

Konopacka Z., 1991 Wpływ narkozy CO_2 i N_2O na wyniki sztucznego unasieniania matek pszczelich. [Effect of CO_2 and N_2O anaesthetics on the results of instrumental insemination of honey bee queens - in Polish]. Pszczelnicze Zeszyty Naukowe 35:3-18.

Kottelat M., Freyhof J., 2007 Handbook of European freshwater fishes. Kottelat, Cornol and Freyhof, Berlin.

Laidlaw H. H., 1944 Artificial insemination of the queen bee (*Apis mellifera* L.): morphological basis and results. Journal of Morphology 74(3):429-465.

Laidlaw H. H., Page R. E., 1997 Queen Rearing and Bee Breeding. Wicwas, Cheschire.

Lazăr S., 2002 Bioecologie şi tehnologie apicolă. Editura Alfa, Iaşi.

Le Conte Y., Page R. E., 2014 Queen and young larval pheromones impact nursing and reproductive physiology of honey bee (*Apis mellifera*) workers. Behavioral Ecology and Sociobiology 12(68):2059-2073.

Mackensen O., 1947 Effect of carbon dioxide on initial oviposition of artificially inseminated and virgin queens bees. Journal of Economic Enotmology 40:344-349.

Mackensen O., 1951 Viability and sex determination in the honey bee (*Apis mellifera* L.). Genetics 36(5):500-509.

Mackensen O., 1964 Relation of semen volume to success in artificial insemination of queen honey bees. Journal of Economic Enotmology 57:581–583.

Mackensen O., 2014 Effect of carbon dioxide on initial oviposition of artificially inseminated and virgin queen bees. Journal of Economic Entomology 40(3):344-349.

Mackensen O., Ruttner F., 1976 Însămânțarea artificială a mătcii. Ediţia a 2-a. Cap. V. Tehnica însămânţării. Editura Apimondia, Bucureşti, pp. 69-86.

Mackensen O., Tucker K. W., 1970 Instrumental Insemination of Queen Bees. Entomology Research Division, Agricultural Research Service.

Mag I. V., Petrescu R. M., Petrescu D. C., 2006 Diversitatea polenului recoltat de himenopterele apoidee – un posibil indicator al sănătăţii pajiştilor. Agricultura. Revistă de Ştiinţă și Practică Agricolă 1-2(57-58):100-103.

Mag-Mureşan I. V., Bud I., 2004 Organismul femel poate influenţa raportul sexual al propriei progenituri la specia *Poecilia reticulata* (Pisces, Poeciliidae). (The female organism can influence the sex-ratio of its own progeny in *Poecilia reticulata* species (Pisces, Poeciliidae)). Studia Universitatis Vasile-Goldis Arad, Seria Stiintele Vietii 14:141-144.

Mason I. L., Mason I. L., (eds), 1984 Honeybees. In: Evolution of domesticated animals. Chapter 65, pp. 403-415. Longman Group, Londra.

Mărghitaş L. A., 2008 Albinele şi produsele lor. Editura Ceres.

Mattila H. R., Seeley T. D., 2007 Genetic diversity in honey bee colonies enhances productivity and fitness. Science 317(5836):362-364.

Moritz R., Southwick E. E., 2012 Bees as superorganisms: an evolutionary reality. Springer Science & Business Media.

Murilhas A. M., 2002 *Varroa destructor* infestation impact on *Apis mellifera carnica* capped worker brood production, bee population and honey storage in a Mediterranean climate. Apidologie 33(3):271-281.

Niño E. L., Malka O., Hefetz A., Teal P., Hayes J., Grozinger C. M., 2012 Effects of honey bee (*Apis mellifera* L.) queen insemination volume on worker behavior and physiology. Journal of Insect Physiology 58:1082–1089.

Niño E. L., Tarpy D. R., Grozinger C. M., 2013 Differential effects of insemination volume and substance on reproductive changes in honey bee queens (*Apis mellifera* L.). Insect Molecular Biology 22:233–244.

Nowak M., Petrescu-Mag I. V., Mierzwa D., Popek W., 2009 On some interesting Romanian gudgeons (Cyprinidae: *Romanogobio*) found in the collection of Museum and Institute of Zoology PAS. AES Bioflux 1(2):81-88.

Odagiu A., Oroian I. G., 2010 Consideraţii ecopatologice la insecte, abordare moleculară. Vol 1. *Apis melifera* L.. Editura Bioflux, Cluj-Napoca. Versiunea online, ISBN 978-606-8191-07-2.

Page R. E., 1980 The evolution of multiple mating behavior by honey bee queens (*Apis mellifera* L.). Genetics 96(1):263-273.

Page R. E. Jr., Gadau J., Beye M., 2002 The emergence of Hymenopteran Genetics. Genetics 160:375–379.

Page R. E., Erickson E. H., 1988 Reproduction by worker honey bees (*Apis mellifera* L.). Behavioral Ecology and Sociobiology 23(2):117-126.

Pătruică S., 2005 Tehnologia creşterii albinelor. Editura Eurobit, Timişoara.

Pătruică S., 2006 Tehnologia creşterii albinelor, Curs universitar. Editura Eurobit, Timişoara.

Pătruică S., Bura M., 2017 Insamantarea artificiala ca metoda de ameliorare a albinelor. Editura Eurobit, Timisoara.

Payne A. N., Rangel J., 2018 The effect of queen insemination volume on the growth of newly established honey bee (*Apis mellifera*) colonies. Apidologie 49(4):1-12.

Parker G. A., 1970 Sperm competition and its evolutionary consequences in the insects. Biological Reviews 45(4):525-567.

Phiancharoen M., Wongsiri S., Koeniger N., Koeniger G., 2004 Instrumental insemination of *Apis mellifera* queens with hetero-and conspecific spermatozoa results in different sperm survival. Apidologie 35:503–511.

Phiancharoen M., Wongsiri S., Hepburn H. R., 2011 Queen production and instrumental insemination of *Apis florea* queens. Apidologie 42(3):307-311.

Raffiudin R., Crozier R. H., 2007 Phylogenetic analysis of honey bee behavioral evolution. Molecular Phylogenetics and Evolution 43(2):543-552.

Requena G. S., Alonzo S. H., 2017 Sperm competition games when males invest in paternal care. In: Proc. R. Soc. B (Vol. 284, No. 1860, p. 20171266). The Royal Society.

Richard F. J., Schal C., Tarpy D. R., Grozinger C. M., 2011 Effects of instrumental insemination and insemination quantity on Dufour's gland chemical profiles and vitellogenin expression in honey bee queens (*Apis mellifera*). Journal of Chemical Ecology 37(9):1027-1036.

Richard F. J., Tarpy D. R., Grozinger C. M., 2007 Effects of insemination quantity on honey bee queen physiology. PLoS One. https://doi.org/10.1371/journal.pone.0000980

Roberts K. E., Evison S. E. F., Baer B., Hughes W. O. H., 2015 The cost of promiscuity: sexual transmission of Nosema microsporidian parasites in polyandrous honey bees. Scientific reports, 5, 10982.

Rosenkranz P., 1999 Honey bee (*Apis mellifera* L.) tolerance to *Varroa jacobsoni* Oud. in South America. Apidologie 30(2-3):159-172.

Ruttner F., 1980 Creșterea mătcilor. Editura Apimondia, București.

Ruttner F., 1976 Insămînţarea artificială a mătcii. Ediţia a 2-a. Apimondia, Bucureşti.

Ruttner F., Maul V., 1983 Experimental analysis of reproductive interspecies isolation of *Apis mellifera* L. and *Apis cerana* Fabricius. Apidologie 14:309–327.

Schneider S. S., Lewis L. A., 2004 The vibration signal, modulatory communication and the organization of labor in honey bees, *Apis mellifera*. Apidologie 35(2):117-131.

Simmons L. W., 2014 Sperm competition. The evolution of insect mating systems. Oxford Univ. Press, Oxford, UK, pp. 181-203.

Simpson G. G., 1961 Principles of animal taxonomy. No publisher.
Snodgrass R. E., 1984 Anatomy of the honey bee. Cornell University Press.
Snodgrass R. E., 1925 Anatomy and Physiology of the Honeybee. Mcgeaw-Hallbook Company, Ics New York And London.
Stockley P., (2004 Sperm competition in mammals. Human Fertility 7(2):91-97.
Tarpy D. R., Nielsen D. I., 2002 Sampling error, effective paternity and estimating the genetic structure of honey bee colonies (Hymenoptera: Apidae). Annals of Entomological Society of America 95:513–528.
Tarpy D. R., Nielsen R., Nielsen D. I., 2004 A scientific note on the revised estimates of effective paternity frequency in *Apis*. Insectes Sociaux 51(2):203–204.
Tănăsoiu I. C., Drăgotoiu D., Marin M., Drăgotoiu T., Diniţă G., 2015 Research on the influence of Eco certificated energo-protein use over the performance of bee families. Agriculture and Agricultural Science Procedia 6:265-271.
Tudor I., 2011 Albinele și albinăritul. Ghidul începătorului. Editura Gramen, București.
Visscher P. K., 1980 Adaptations of honey bees (*Apis mellifera*) to problems of nest hygiene. Sociobiology 5(3):249-260.
Visscher P. K., 1989 A quantitative study of worker reproduction in honey bee colonies. Behavioral Ecology and Sociobiology 25(4):247-254.
Wilde J., 1994 Comparison of the development and productivity of bee colonies with naturally and instrumentally inseminated queens kept in different conditions before and after the insemination. Zootechnica 39:135–152.
Wiley E. O., 1978 The evolutionary species concept reconsidered. Systematic zoology, 27(1):17-26.
Wiley E. O., Mayden R. L., 2000 The evolutionary species concept. In: Species concepts and phylogenetic theory. Wheeler Q. D., Meier R. (eds), pp. 70-89, Columbia University Press, New York.
Winston M. L., 1987 The Biology of the Honey Bee. Harvard University Press, Cambridge.
Winston M. L., 1991 The Biology of the Honey Bee. Harvard University Press, Cambridge.
Woyke J., 1962 Natural and artificial insemination of queen honeybees. Bee World 43(1):21-25.
Woyke J., 1973 Instrumental insemination of *Apis cerana indica* queen. Journal of Apicultural Research 12:151–158
Woyke J., 1975 Natural and instrumental insemination of *Apis cerana indica* in India Journal of Apicultural Research 14:153–159.
Woyke J., 1989 Results of instrumental insemination. In: Moritz R.F.A. (Ed.), The Instrumental Insemination of the Queen Bee. Bucharest, Apimondia, pp. 93–103.
Woyke J., 1993 Rearing and instrumental insemination of *Apis florea* queens, in: Asian Apiculture, Proc.1st Int. Conf. on Asian honey bees and bee mites. Bangkok, Thailand, Wicwas Press Cheshire, CT, USA, pp. 206-210.
Woyke J., 2008 Why the eversion of the endophallus of honey bee drone stops at the partly everted stage and significance of this. Apidologie 39(6):627-636.
Woyke J., Wilde J., Wilde M., 2001 *Apis dorsata* drone flights, collection of semen from everted endophalli and instrumental insemination of queens. Apidologie 32:407–416.
Zeuner F. E., 1963 A history of domesticated animals. No publisher.
*** European Comission, 2018 Agriculture and Rural Development. Available online at: https://ec.europa.eu/agriculture/markets_en [Accessed: 22.08.2018].
*** Manualul Apicultorului, 2007 ediția a IX-a, Asociația Crescătorilor de Albine din România, București.
*** Schley – Insemination Instruments, 2018 Available online at: http://www.besamungsgeraet.de/__en/insemination-instruments/ [Accessed: 20.08.2018]

Received: 10 October 2018. Accepted: 21 November 2018. Published online: 01 December 2018.

Authors:
Remus Octav Stoian, University of Oradea, Doctoral School of Engineering, Universitatii Street no 1, Oradea, Romania, e-mail: remusoctav@yahoo.com
Miklos Botha, SC Bioflx SRL, Romania, Cluj-Napoca, 400488, 54 Ceahlau Street, e-mail: miklosbotha@yahoo.com
Ioan Valentin Petrescu-Mag, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Agriculture, Romania, Cluj-Napoca, 400372, 3-5 Calea Manastur; University of Oradea, Doctoral School of Engineering, Romania, Oradea, Universitatii Street no. 1, e-mail: zoobiomag2004@yahoo.com

This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

How to cite this article:
Stoian R. O., Botha M., Petrescu-Mag I. V., 2018 Beekeeping in Romania and artificial insemination of honey bee, *Apis mellifera*. State of the art. ABAH Bioflux 10(2):93-121.