ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2020; 25(4): 1776-1780
doi: 10.25083/rbl/25.4/1776.1780

Received for publication, Received, October, 4, 2019
Accepted, April, 20, 2020

Original paper

First report on the effect of Meloe spp. larvae invasion on Apis mellifera carpathica bees in some apiaries in Romania

ION RADOI[1], FLORENTIN MILEA[1], VIORICA LAGUNOVSCHI LUCHIAN[1], AGRIPINA ȘAPCALIU[2], DAN BODESCU[3], VECDI DEMIRCAN[4]

[1]University of Agronomic Sciences and Veterinary Medicine Bucharest, 050097, Bucharest, Romania
[2]Beekeeping Research and Development Institute, 013975, Bucharest, Romania
[3]University of Agronomical Sciences and Veterinary Medicine Iasi, Romania
[4]Departament of Agricultural Economics, Faculty of Agriculture, Isparta, Turkey

Abstract During the program monitoring bees' health state it was possible to detect an invasion of triungulin larvae of *Meloe spp.* against melliferous bees (*Apis mellifera carpathica*) in apiaries from the South and East regions of the country. We evaluated 35 apiaries (550 bee colonies) located in all the regions of the country, and found the presence of triungulinosa in 8 of them (22.86%). A number of 173 bee colonies have been affected (31.45%), while 54 colonies (9.81%) registered massive depopulations. Evaluation of the melliferous flora in the areas adjacent to the apiaries showed a considerable number of triungulin larvae on the florescence of the plants in the South-East area. Bees' infestation with triungulin larvae of Meloe spp. was diagnosed as singular morbid entity in six apiaries (five in the East and one in the South), while in the other two infested apiaries, the presence of the triungulin larvae was accompanied by other morbid entities, such as the nosemosis. To our knowledge, this is the first report of an attack of the triungulin larvae against *Apis mellifera carpathica* colonies in Romania.

Keywords *Apis mellifera carpathica*, *Meloe spp*., predators, triungulinosis.

To cite this article: RADOI I, MILEA F, LUCHIAN V, ȘAPCALIU A, BODESCU D, DEMIRCAN V. First report on the effect of *Meloe spp.* larvae invasion on *Apis mellifera carpathica* bees in some apiaries in Romania. *Rom Biotechnol Lett*. 2020; 25(4): 1776-1780. DOI: 10.25083/rbl/25.4/1776.1780

✉ *Corresponding author: VIORICA LAGUNOVSCHI LUCHIAN, University of Agronomic Sciences and Veterinary Medicine Bucharest, 050097, Bucharest, Romania
E-mail: vluchian@hotmail.com

Introduction

Triungulinosa is an invasive predators in adult bees (foragers and nurses) caused by the larvae of the coleoptera *Meloe spp.* (blister beetles or oil beetles), encountered during active foraging season (March-September) (TOPOLSKA & al [13]; GHONEIM & al [8]; TOPITZHOFER & al [12]);

Genus Meloe (Coleoptera: Meloidae) has approximately 155 species included in 16 subgenera, with a widespread range all over the world (BOLOGNA & al [2]; Di Giulio & al. [5]); frequently populate forests, orchards and hay fields (CASSOLA & al [4]; ASIMINEI & al [1]). As a rule, adults have a predominantly daytime activity, lack flying capacity and are phytophagous, eating flowers and leaves of *Amaranthaceae*, *Compositae*, *Leguminoasae* and *Solanaceae* plants (GHAHARI & al [7]); These are harmless for the bee colonies, but their larvae are dangerous to bees, because they are phoretic (phoresia is the phenomenon by which an animal attaches to another animal to be transported from one place to another in search of a food source (BOLOGNA & al [2]; BOLOGNA & al [3]; TUTANKHAMUN [14]);. The biological cycle is hypermetamorphic, presenting considerable morphological differences during successive larval development stages (TOPOLSKA & al [13]). Triungulin larvae are long (1-5 mm), have three pairs of legs with claws with which they swiftly climb leaves and melliferous flowers and onward to the bodies of adult foraging bees (BOLOGNA & al [2]; BOLOGNA & al [3]). The oral apparatus of the larvae enables them to perforate the body of the worker bee during foraging (at the level of intersegmental membranes), after which they penetrate with half of their bodies in the bee's abdomen (PINTO & al [10]; SAVU & al [11]), attack the bee's nervous system, feed on the bee's haemolymph (TOPOLSKA & al [13]), causing unrest, agitation, convulsions and loss of flight capacity. When they reach the hive through foraging bees, the *Meloe spp.* larvae migrate from the bees' body and devour the eggs, the brood, and the nectar and pollen reserves from the hive (TOPOLSKA & al [13]; SAVU & al [11]; TOPITZHOFER & al [12]; ASIMINEI & al [1]). Bees' infestation with triungulin larvae occurs upon pollen foraging, the contamination sources are the melliferous plants in the areas where blister beetles live (SAVU & al [11]). Although blister beetles have a cosmopolitan distribution, literature data on the invasion and effects of the triungulin larvae on the domestic bee colonies is reduced (TOPITZHOFER & al [12]).

The purpose of this study is to detect the presence of *Meloe spp.* larvae and the effects of their presence on the bee colonies in various areas of the country.

Materials and Methods

Investigations took place during the active beekeeping season (March-September) 2018. We examined 35 apiaries in various geographical areas of Romania, totalling 2527 bee colonies. The distribution of the apiaries has been the following: three apiaries were located in the North area, one apiary in the West, 18 apiaries in the South and 13 apiaries in the East. In each apiary, all bee colonies were visually inspected. In some apiaries we found dead bees and bees with altered behaviour. In the plastic flacons, we collected random dead and agonizing bees for additional investigations. Additionally, when beekeepers handled the frames with bees from the hive, yellow-brownish larvae were noticed on the beekeepers' hands, they were collected in plastic tubes for future examination. The lab investigation methodology to diagnose bee diseases developed according to protocols of *World Organisation for Animal Health*, 2018 (OIE, 2018). Also, melliferous plants in the proximity of the apiaries were scrutinized to detect potential triungulin larvae kleptoparasites of bees.

Results and Discussion

During the inspection of each hive in the 35 evaluated apiaries (2527 bee colonies), in some bee colonies we noticed bees that presented a modified behaviour. Sick bees presented the dehydration syndrome, spasmodic and violently convulsive movements. At the hive entrance, we noticed paralysed bees or bees incapable of flying, as well as remnants of devoured bee eggs. Furthermore, we also identified dead bees. After a close inspection of the bees on the hive frames, we noticed that the bees were carrying some yellow-brownish larvae (Figure 1 and Figure 2).

Figure 1. Adult bee parasitized by *Meloe spp.* larvae in an apiary from the East area (Pathology Laboratory, BRDIB) (Nikon SMZ645 × 10 magnification).

Figure 2. Worker bees parasitized by *Meloe spp.* larvae on the hive frames (Photo by beekeeper D.V, East area).

The larvae load was three to 34 larvae/bee. The larvae that we noticed on the live bees were hiding between the abdominal tergite, and by a light compression of the bee abdomen, they jumped to the beekeeper's or examiner's hand. The larvae (collected off bees' body or beekeeper's hand) were examined individually under the stereo microscope and morphologically identified based on previous descriptions (BOLOGNA & al [2]; DI GIULIO & al [6]) as being the triungulin larvae of *Meloe spp.* The larvae have a long fusiform body, yellow-brownish in colour, two to three mm long and 0.4-0.6 mm wide, with the thorax and abdomen tergite and sternite well sclerotized (Figure 3).

Figure 3. *Meloe spp.* larva (Pathology Laboratory, BRDIB, Nikon SMZ645, × 10 magnification).

Of the total of examined apiaries (n=35), eight (22.86%) were infested with triungulin larvae, being located in the South (n=3) and East (n=5) of the country. The situation as registered in the eight apiaries in which the disease was present is covered by Table 1. We noticed that 31.45% (173/550) bee colonies were affected, and in 9.81% (54/550) bee colonies massive depopulations were registered. Bees' infestation with triungulin larvae of *Meloe spp.* was diagnosed as singular morbid entity in six apiaries (five in the East and one in the South), while in the other two infested apiaries, the presence of the triungulin larvae was accompanied by other morbid entities, such as the nosemosis.

The total samples of eight apiaries affected by the parasite were distributed in Southern (three apiaries) and Eastern Romania (five apiaries). However, there is an important feature of the parasite infection pattern in the two regions in terms of the parasite sources. Table 1 presents the raw data we gathered, while Table 2, 3 and 4 present the descriptive statistics (averages, variances, standard deviations and confidence intervals) of the total eight apiaries sample, of the three apiaries in Southern Romania and of the five apiaries in Eastern Romania, respectively.

We admit however that given the low number of investigated apiaries we cannot support statistically such an inference and a further more extended study is needed in this direction.

Table 1. Distribution of apiaries and number of bee colonies infested with triungulin larva of *Meloe spp.* and type of melliferous plant

Origin area of the apiary	Number of bee colonies monitored	Number of bee colonies affected	Number of bee colonies with massive depopulations
South (apiary 1)	64	26	3
South (apiary 2)	75	15	8
South (apiary 3)	24	11	0
East (apiary 4)	92	45	0
East (apiary 5)	46	46	40
East (apiary 6)	25	5	0
East (apiary 7)	200	20	3
East (apiary 8)	24	5	0
Total colonies	**550**	**173**	**54**

Table 2. Descriptive statistics parameters of the total sample formed by the eight (**8**) apiaries in Southern and Eastern Romania

	Number of bee colonies monitored	Number of bee colonies affected	Percentage of bee colonies affected	Number of bee colonies with massive depopulation	Percentage of bee colonies with massive depopulation	Percentage of bee colonies with respect to the affected colonies
Mean values	68.75	21.63	38.28	6.75	0.13	0.21
Variance	3466.50	267.41	822.49	188.21	0.09	0.10
Standard deviation	55.07	15.30	26.83	12.83	0.28	0.30
Confidence interval – normal distribution (95% confidence level)	68.75±38.16 [30.59; 106.91]	21.63±10.60 [11.03; 32.23]	38.28±18.59 [19.63; 56.87]	6.75±8.89 N/A	0.13±0.20 N/A	0.21±0.21
Confidence interval – T-student distribution (8 degree, 95% confidence level)	68.75±26.45 [42.23 ;95.2]	21.63±7.35 [14.23; 28.98]	38.28±12.88 [25.40; 51.16]	6.75±6.16 [0.59; 12.91]	0.14±0.14 [0.00; 0.27]	0.21±0.14 [0.07; 0.35]

Table 3. Descriptive statistics parameters of the partial sample formed by the **3** apiaries in Southern Romania

	Number of bee colonies monitored	**Number of bee colonies affected**	**Percentage of bee colonies affected**	**Number of bee colonies with massive depopulation**	**Percentage of bee colonies with massive depopulation**	**Percentage of bee colonies with respect to the affected colonies**
Mean values	20.38	6.50	13.31	1.38	0.02	0.08
Variance	720.33	60.33	186.65	16.33	0.00	0.08
Standard deviation	21.91	6.34	11.15	3.30	0.04	0.23
Confidence interval - normal distribution (95% confidence level)	20.38±15.19	6.50±4.39	13.31±7.73	1.38±2.29	0.02±0.03	0.08±0.16
Confidence interval – T-student distribution (3 degree, 95% confidence level)	20.38±10.52	6.50±3.05	13.31±5.36	1.38±1.58	0.02±0.02	0.08±0.11

Table 4. Descriptive statistics parameters of the partial sample formed by the **5** apiaries in Eastern Romania

	Number of bee colonies monitored	**Number of bee colonies affected**	**Percentage of bee colonies affected**	**Number of bee colonies with massive depopulation**	**Percentage of bee colonies with massive depopulation**	**Percentage of bee colonies with respect to the affected colonies**
Mean values	48.38	15.13	24.97	5.38	0.11	0.13
Variance	5456.80	415.70	1336.70	309.80	0.15	0.14
Standard deviation	66.07	18.24	32.70	15.74	0.35	0.34
Confidence interval - normal distribution	48.38±45.78	15.13±12.64	24.97±22.66	5.38±10.91	0.11±0.24	0.13±0.23
Confidence interval - T-Student distribution (5 degree, 95% confidence level)	48.38±31.73	15.13±8.76	24.97±15.70	5.38±7.56	0.11±0.17	0.13±0.16

Discussion

The triungulin larvae of *Meloe* spp. are predators and kleptoparasites of bees, feeding on bee eggs and larvae, stored nectar and pollen (TOPITZHOFER & al [12]), causing sometimes important damages in the apiaries they reach. To our knowledge, this is the first report on massive infestation of *Apis meliffera carpathica* colonies with triungulin larvae of *Meloe spp.* in Romania. Part of the infested bee colonies presented massive depopulations as result of the death of the worker bees, nurses and brood, as well as result of parasitized adult bees' abandoning the hive. Additionally, the reduction in hive population occurred subsequently after the queen ceased activity, as well as after their being devoured by triungulin larvae, a typical behaviour of the parasites (GHONEIM [8]). Historically, we conclude that the adult bugs of *Meloe spp.* were noticed by beekeepers in the ground under the hive, thus contributing in maintaining the infestation in that area.

During these investigations, significant economic losses were registered for the beekeeping in the South-East area of the country, as a result of the serious impact upon the bee colonies from massive depopulations and bee products reduction which caused worry among beekeepers and imposed the need to urgently apply prophylactic and combat measures against *Meloe spp.* Antiparasitic measures taken in the affected hives registered positive results. Thus, anti-acariens treatments with veterinary bee-tolerated products were applied (Varachet Forte, SC ICDA Bucharest, Romania), in addition to measures taken to stop the development of adult bugs on the ground in the apiary yard (soil was treated with anhydrous lime powder). Previous studies showed that mortality rate among bees parasitized with *Meloe spp.* larvae dropped by 32% in 24 hours after low amounts of naphthalene were placed on the hive bottom. Additionally, periodical change of the apiary yard represented one of the most efficient measures to prevent the infestation of bee colonies with *Meloe spp.* larvae, which also involves avoiding the plants that are incriminated for favouring triungulin larvae development.

Conclusion

To conclude, we have described here the infestation of *Apis mellifera charpatica* bee colonies with larvae of the Coleoptera order insect, *Meloe spp*. in the South and South-East areas of the country, this being the first report detecting an attack with triungulin larvae in the territory of Romania. At the same time, this study intends to set the alarm on the effects that this insect from the Coleoptera order has on the health and viability of the bee colonies, informing actions and permanent professional trainings being necessary among beekeepers and specialists in the field. Additional studies are necessary and should provide

more information on the real spread of the *Meloe spp*. parasites in the bee populations of our country, as well as to identify some new prophylaxis measures and control methods against this morbid entity.

Acknowledgements

Preliminary results of PhD thesis with title: "*Exploiting and assessing the antimicotics potential of plant extracts in bees' fungal diseases prevention*".

References

1. ASIMINEI S., SOLCAN G., SECAȘIU V., MITROIU M.D., PUCHIANU G., ISAN E., ANDERCO Ș., DOBRE G. Patologia albinei melifere. Ed. "Ion Ionescu de la Brad", Iași (2016).
2. BOLOGNA M.A., PINTO J.D. Phylogenetic studies of Meloidae (Coleoptera), with emphasis on the evolution of phoresy. *Syst Entomol* 26 (1), 33-72 (2001).
3. BOLOGNA M.A., PINTO J.D. The Old World genera of Meloidae (Coleoptera): a key and synopsis. Journal of Natural History 36, 2013-2102 (2002).
4. CASSOLA F., JASKUŁA R. Materials to the knowledge of the tiger beetles of Romania (Coleoptera Cicindelidae). *Polish Journal of Entomology* 73, 193-214 (2004).
5. DI GIULIO A., SCIOTI A., BOLOGNA M.A. Revision of first instar larvae of Meloe, subgenera Eurymeloe and Coelomeloe, with new descriptions and a key to the species (Coleoptera: Meloidae). *Ital J Zool* 80, 242-254 (2013).
6. DI GIULIO A., CAROSI M., KHODAPARAST R., BOLOGNA M.A. Morphology of a new blister beetle (Coleoptera, Meloidae) larval type challenges the evolutionary trends of phoresy-related characters in the genus Meloe. *Entomologia* 2, 164 (2014).
7. GHAHARI H., CAMPOS-SOLDINI M.P. An annotated catalogue of blister-beetles (Coleptera: Tenebrionoidea: Meloidae) of Iran. *Entomofauna* 40 (5), 59-138 (2019).
8. GHONEIM K.S. Global zoogeography and systematic approaches of the blister beetles (Coleoptera: Meloidae): a bibliographic review. *IJRBS* 2 (3), 1-45 (2013).
9. OIE-WORLD Organisation for Animal Health, OIE-Manual of diagnostic tests and vaccines for terrestrial animal. Section 3.2. Apinae, 711-782 (2018).
10. PINTO J.D., SELANDER R.B. The bionomics of blister beetles of the genus Meloe and a classification of the New World species (Biological monographs). University of Illinois Press, Illinois (1970).
11. SAVU V., ȘAPCALIU A. Patologia albinelor. Editura Fundației România de Mâine, București (in romanian) (2013).
12. TOPITZHOFER E., MARSHALL CH., ROYCE L., SAGILI R. First published report of triungulin larvae of Meloe spp. (Coleoptera: Meloidae) on honey bees in Oregon, U.S.A. *Pan-Pac Entomol* 94 (3), 163-166 (2018).
13. TOPOLSKA G., HARTWIG A., KRZYZAŃSKA K. Triungulin larvae of Meloe variegatus Donov (Coleoptera: Meloidae); morphology, biology and an incident of apiary infestation. *Wiad Parazytol* 47 (1), 115-117 (2001).
14. TUTANKHAMUN S.A. Sexual ecological behavioral phases varied among blister beetles (Coleoptera: Meloidae). *Int J Account Audit Taxation* 3 (1), 243-257 (2016).