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The accompanying fauna of *Osmia cornuta* and *Osmia rufa* and effective measures of protection

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Abstract

The present paper treats species of the fauna accompanying *Osmia cornuta* (Latreille) and *O. rufa* (L.), with special attention to measures of protection against the most significant organisms that restrict their populations. The most significant organisms that restrict *Osmia* populations are the fly *Cacoxenus indagator* Loew and the mite *Chaetodactylus osmiae* Dufour. The wasp *Monodontomerus obscurus* Westwood is a significant organism that restricts *Osmia* populations in paper tubes used as nesting material, and is found sporadically in marsh reeds used for this purpose in the vicinity of Belgrade. However, it was not present in lamellar boxes. *Anthrax anthrax* Schrank is an important organism restricting populations of *O. cornuta* and *O. rufa* in several localities in the vicinity of Pisa (Italy). The other species of the accompanying fauna exerted no significant influence on the abundance of populations of these bees in the vicinity of Belgrade. Species of the accompanying fauna are grouped in the following categories: cleptoparasites, parasitoids, nest destroyers, predators, cleptobionts, and accidental nest residents.

Key words: orchard bees, *Osmia cornuta*, *Osmia rufa*, accompanying fauna, parasites, parasitoids.

Introduction

The use of *Osmia* for pollination of orchards was first attempted during the second half of the last century in Japan with the species *Osmia cornifrons* (Radoszkowski) (Maeta and Kitamura, 1964, 1965). From the very beginning, satisfactory results were obtained in using these species as orchard pollinators, and the practice has been continued and advanced down to the present day. Today the majority of apple orchards in Japan are pollinated with the aid of *Osmia* (Maeta, 1978; Sekita, 2001). Encouraged by the successful commercialisation of *Megachile rotundata* (F.), and by the results achieved by Japanese authors with their species of *Osmia*, investigators in the United States during the 1970's and 1980's started to investigate the American native species *Osmia lignaria* Say as a pollinator of orchards (Torchio, 1976; Bosch and Kemp, 2001), somewhat later examining the possibility of using a species introduced from Japan (*O. cornifrons*) for this purpose (Batra, 1998). At the same time, investigations were initiated on the possibility of using as pollinators *Osmia rufa* (L.) in Denmark (Holm, 1973; Kristjansson, 1989), England (Raw, 1972), and *Osmia cornuta* (Latreille) in Spain (Bosch, 1994), Serbia (Krunić *et al*., 1995, 2001), and Italy (Pinzauti, 1992; Felicioli, 1995; Ladurner *et al*., 2002; Maccagnani *et al*., 2003). The technology of raising *O. cornuta* is known today, and this bee can be reared in the desired numbers (Stanisavljević, 2000).

Many parasitoids, predators, nest destroyers, and accidental nest residents can be found in the nests of *O. cornuta* and *O. rufa* in Southeastern Europe. With the increase in bee populations, the accompanying fauna also increases in both diversity and abundance. For management and multiplication of populations of *O. cornuta* and *O. rufa*, control of certain species of the accompanying fauna is essential, both during the period of activity of the bees and during the periods of their development and overwintering. There are various measures of protection against the accompanying fauna, some of which depend upon the type of nesting material used. For example, cocoons of *O. cornuta* and *O. rufa* in paper tubes are often parasitized by the wasp *Monodontomerus obscurus* Westwood. Insectivorous birds find it easy to extract paper tubes with cocoons from wooden blocks. Birds and mice destroy tubes on the ground and eat the cocoons inside. In this way, in a short time birds can destroy a large part not only of bee populations in paper tubes, but also of populations in reeds (figure 1). They can destroy easily bee populations before and during overwintering if the reeds are not adequately protected. Nests of *O. cornuta* and *O. rufa* in lamellar boxes are better protected against some parasitoids and birds, but they often house the coleopteran *Trichodes apiarius* L., the mite *Chaetodactylus osmiae* Dufour, the fly *Anthrax anthrax* Schrank, certain Dermestidae, and other species of the accompanying fauna.

Figure 1. Reed nest destroyed by birds.
(In colour at www.bulletinofinsectology.org)

Of all species of the accompanying fauna, the most significant organisms that restrict populations of *O. cornuta* and *O. rufa* in Southeast Europe are the dipteran *Cacoxenus indagator* Loew and the mite *Ch. osmiae* (Krunić *et al*., 1995; Stanisavljević, 1996; Krunić *et al*., 1999). In several localities around Pisa (Italy) a significant organism that restricts *Osmia* populations is the dipteran *A. anthrax* (Felicioli, 2000), which is very rare in populations in the vicinity of Belgrade. The abundance and diversity of individual members of the accompanying fauna vary from one locality to the next, as well as from season to season. Apart from fungal diseases caused by spores of *Ascosphaera* spp. (so-called chalkbrood), other diseases of *O. cornuta* and *O. rufa* are unknown to date. In populations in the vicinity of Belgrade, we had no significant presence of fungal diseases caused by *Ascosphaera* spp., probably because 80% of the nesting material used in every season consisted of previously unused reeds, while reeds that had been used during the previous season represented only 20%. Spores of fungal diseases are resistant and persist for years in used nesting material. Because of the danger of fungal diseases and *Ch. osmiae*, lamellar boxes that are used every year should be subjected to high temperature or treatment with a 0.07% solution of endosulfan before use (Krunić *et al*., 2001). It is recommendable to burn reeds used for several seasons after emergence of the bees.

The following species of the accompanying fauna were registered in populations of *O. cornuta* and *O. rufa* in Southeastern Europe, they are grouped in the following categories: cleptoparasites, parasitoids, nest destroyers, predators, cleptobionts, and accidental nest residents as reported in a previously published paper (Krunić *et al*., 1995).

Cleptoparasites

Cacoxenus indagator Loew (Diptera Drosophilidae). This species is distributed on the continent of Europe (Julliard, 1947; Kekić, 2002).

Adults of *C. indagator* are 3 to 3.5 mm long. The thorax is light-grey, the abdomen black with light transverse bands. The large eyes are brown to dirty-red in colour (figure 2). Females of *C. indagator* lay their eggs on the pollen provisions in nests of *O. cornuta* and *O. rufa*. Larvae develop from the eggs and feed actively until they reach the overwintering prepupal stage (figure 3). With the increase of daily temperatures in spring, barrel-shaped pupae develop from the prepupae over a period of several days. Adults appear after a relatively short duration of the pupal stage (about 22 days at 20 - 25 °C) (Stanisavljević, 1996). The last instar larvae of this fly are able to migrate from the internal cells to the vestibule of the tunnel by perforating the mud partitions with their mouth parts. Due to this larval behaviour it is often possible to observe large amounts of larvae or pupae (20 to 40) in the vestibule of the nest (Julliard, 1948) (figure 4). Earlier emergence of bees from the nest, which is accompanied by perforation of its partitions, facilitates the subsequent unhindered departure of *C. indagator* adults. The length of the active period of adults of *C. indagator* is synchronized with the completion of the active period of *O. cornuta* (Stanisavljević, 1996).

C. indagator is very frequent in populations of *O. cornuta* and *O. rufa* in the wider area of Belgrade. In nature it usually appears 2-3 weeks after the appearance of the first individual orchard bees, i.e., at the end of March

Figure 2. *C. indagator*, female.
(In colour at www.bulletinofinsectology.org)

Figure 3. *C. indagator*, overwintering prepupal stage.
(In colour at www.bulletinofinsectology.org)

Figure 4. *C. indagator*, large amount of larvae or pupae in the vestibule of the *O. rufa* nest.
(In colour at www.bulletinofinsectology.org)

or in April. At the beginning, males and females of *C. indagator* can be seen in the shelter, but only females remain after mating, which lasts up to 10 days (Stanisavljević, 1996). The females appear to be continuously on "guard duty" at the side of a reed near the opening of a bee nest (figure 5). When a female bee leaves the nest, the fly hurriedly enters it, lays an egg on the pollen provision, rapidly departs, and awaits the next opportunity (Julliard, 1947, 1948; Coutin and Desmier de Chenon, 1983; Stanisavljević, 1996). Sometimes a female bee surprises a *C. indagator* female in its nest. Being considerably smaller than the bee, the female fly readily abandons the nest in this case. Females of *O. cornuta* dive frequently on females of *C. indagator*, but this has no effect on the protection of their nests. The placing of strips of sticky paper on bundles at a certain distance from the reed openings, where *C. indagator* females usually wait for bees to leave their nests, did not yield significant results in controlling this cleptoparasite. Only limited numbers of *C. indagator* individuals got stuck on the paper (Stanisavljević, 1996). Individuals of *C. indagator* are accustomed to mass flight of orchard bees, and in most cases they do not react to their movement. Such behavior allows them to be collected with an aspirator, during which they make no sudden attempt to escape. This method yielded satisfactory results for the control of this cleptoparasite in multiplication shelters (Krunić *et al*., 2001). Such a method of removal cannot be used in nests disposed in orchards because it would entail a great loss of time with a negligible result. However, when *C. indagator* populations in multiplication shelters were removed with an aspirator in successive years, then their abundance in the whole managed bee population was considerably reduced (Stanisavljević, 1996). The number of larvae of *C. indagator* in nest cells varies. When only two or three larvae of the cleptoparasite are present in the nest, the bee larva will develop alongside them and spin a cocoon, that is noticeably smaller than usual (Juillard, 1948; Raw, 1972; Stanisavljević, 1996). At some localities, we found 10-20 tiny larvae of *C. indagator* in certain cells of *O. cornuta*. In such circumstances, the bee larva has no chance to develop. When present in large numbers, *C. indagator* larvae can perforate the wall of the neighboring cell or of several cells in a row and consume their contents, too. The number of cells infested with *C. indagator* varied significantly from one locality to the next and from year to year. Without mechanical control, this cleptoparasite can become very abundant in nests in the area of Belgrade and significantly reduce multiplication of populations of *O. cornuta* and *O. rufa*.

Populations of *O. cornuta* reared in almond orchards in Spain were not infested or negligibly infested with *C. indagator*, because bees in those orchards for the most part complete their activity before the hatching of these cleptoparasites (Bosch, 1992). In Italy, wild populations of *O. cornuta* are infested at low levels, while *O. rufa* populations can show high levels of *C. indagator* infestation due to the differences in the flying season of the two bee species. Both *O. cornuta* and *O. rufa* managed populations, if released in April, showed high levels of infestation if the nests of the orchard bees were not renewed every year or if the *C. indagator* adults weren't captured (Felicioli, 2000).

Figure 5. *C. indagator*, female at the side of a reed near the openings of bee nests.
(In colour at www.bulletinofinsectology.org)

Chaetodactylus osmiae Dufour (Acarina Chaetodactylidae). This mite is distributed in Europe as far north as Denmark, as far as the Urals in the east, to the southern limits of the Balkan Peninsula in the south, and as far as the Atlantic Coast (including Great Britain) in the west. It is found in the nests of many European species of solitary bees (Černy and Samšiňák, 1971).

Mites of the genus *Chaetodactylus* have very similar development, with two reproductive cycles that repeat themselves periodically (Zachvatkin, 1941).

The direct cycle consists of the following stages: egg, larva, protonymph, tritonymph, adult (male and female). This cycle appears when the bee nest contains abundance of food (pollen and nectar). The nest then most often also contains enough moisture for the direct development of the mites. In this case, the development cycle is short and can repeat itself as many as ten times in a single season. The number of cycles depends exclusively on the amount of pollen and nectar, moisture content of the surroundings and temperature (Stanisavljević, 1996).

The indirect cycle differs from the direct cycle in the appearance of a very resistant stage that enters dormancy, the so-called hypopus (figure 6, a and b). The hypopus that appears sporadically in the life cycle of mites of the order Astigmata, is also called a heteromorphic deutonymph, while the tritonymph is called a homeomorphic deutonymph. The hypopus occurs in two basic forms, mobile and immobile (encysted) (Krombein, 1962; Fain, 1966). The indirect cycle unfolds according to the following scheme: egg, larva, protonymph, hypopus (mobile or immobile), tritonymph, adult. This type of cycle (facultative hypopodia) sets in when environmental conditions are unfavorable for development of the direct cycle, i.e., when there is not enough moisture and food in the cell. The hypopus is a supplementary stage between the protonymph and tritonymph stage and in fact represents the deutonymph stage. A characteristic distinction of this facultative stage in the development of all mites of the order Astigmata (and of *Ch. osmiae*) in relation to the deutonymphs of other mites is that it lacks a mouth and mouth parts and does not feed, but rather exists at the

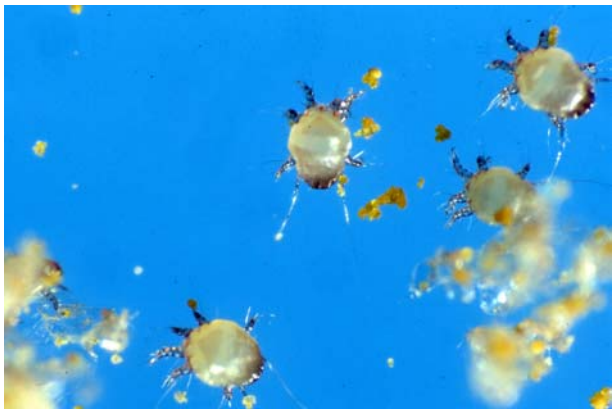

Figure 6a. *Ch. osmiae*, mobile hypopi.
(In colour at www.bulletinofinsectology.org)

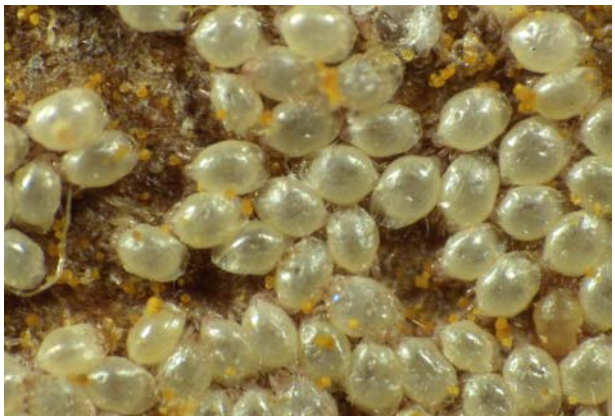

Figure 6b. *Ch. osmiae*, immobile hypopi.
(In colour at www.bulletinofinsectology.org)

expense of reserves accumulated in the preceding stage. The hypopus represents a resistant form which ensures survival under the unfavorable environmental conditions that occur in bee nests at the end of the summer and throughout the winter. It also ensures dispersal of the species.

The existence of two types of hypopus is an adaptation linked to dispersal and preservation of the species. To be specific, the mobile type of hypopus possesses four pairs of well-developed legs with specialized hook-like outgrowths that enable it to attach itself easily to adult bees (figure 7). The immobile type of hypopus has short legs, and its parts for attachment are very rudimentary (figure 6b). They do not emerge from the skin (exuviae) of the protonymph until the moment of their activation, i.e., transformation into tritonymphs (Stanisavljević, 1996).

Mobile and immobile hypopi are of virtually equal significance for the survival of this species of mite. The mobile type of hypopus is significant for dispersal and transfer. Its development cycle is synchronized with the development cycle of the bees. This is the form of the mite that is transferred from the old to the new nest, i.e., from one generation of bees to their progeny by means of the bee as a host, which carries them on its body at the time of emergence. Encysted hypopi always remain

Figure 7. *Ch. osmiae* on *O. cornuta* female.
(In colour at www.bulletinofinsectology.org)

in the old nest of the host bee. This encysted form of mite awaits the arrival of a bee of the same or some other species that will take over the old abandoned nest. The immobile type of hypopus has the primary role of preservation of the species under unfavorable conditions, since it is capable of remaining in the dormant state for several years until conditions for its activation are achieved (Seidelmann, 1990). Under favorable conditions, it is activated and develops into a tritonymph, which develops into an adult female.

When transported into a cell of the host, a mobile hypopus continues its development cycle. It is transformed into a tritonymph, which is very active, feeds at an accelerated rate, and rapidly develops into an adult female. Females of *Ch. osmiae* lay eggs from which males develop very rapidly. They mate with their mothers or with other females found in the same cell. After mating, the females lay eggs from which the complete (direct) cycle proceeds and repeats itself, with the result that a large colony of mites is soon formed.

Under the climatic conditions of the Southeastern Europe, encysted hypopi appear from the middle of August onward, while the first mobile hypopi appear at the end of August (Stanisavljević, 1996).

According to some authors (Van Lith, 1957; Krombein, 1962; Seidelmann, 1990), mites of the genus *Chaetodactylus* sometimes consume the contents of bee eggs and even certain later stages of development in addition to pollen and nectar. These mites are incapable of perforating the wall of the bee's cocoon. In certain cells, we found a tiny cocoon with a developed bee inside and a multitude of mite hypopi around it. Under such conditions, the bee's cocoon is considerably reduced in size due to a shortage of food during larval development (Stanisavljević, 1996).

Male and female bees during emergence from the nest pass through cells with many mobile mite hypopi, which they carry with them in sometimes great numbers. Certain females can be completely yellow from the multitude of hypopi on their bodies (figure 7). The hypopi undergo mass multiplication if they come across pollen in the nest cell. When the female bee closes a cell infested with hypopi, they most often fill all of the space in it. If reeds for bee nesting were used several years in

a row, then infestation with the mite *Ch. osmiae* in our populations at certain localities attained more than 50% of established cells (Krunić *et al*., 2001). If cocoons are infested with these mites, it is useful to disinfect them with endosulfan (a 0.007% aqueous solution) before taking them into the field. Treating cocoons of *O. cornuta* and *O. rufa* during the winter period with a 0.007% solution of endosulfan for a period of 3 min is a very effective method of controlling all stages in development of the mite *Ch. osmiae*. It was found that such treatment of cocoons had no negative effect on the bees inside (Krunić *et al*., 2001). In Japan, endosulfan is directly sprayed on empty and already inhabited reeds to control the mite *Chaetodactylus nipponicus* Kurosa, which greatly reduces populations of the bee *O. cornifrons*. If *Osmia* cocoons are not stripped from the reeds, there will always remain a certain percentage of mites in them that survive this treatment (Yamada, 1990; Sekita and Yamada, 1993). For this reason, stripping of cocoons from the nesting material and treating them with endosulfan is a much more effective way of controlling this cleptoparasite.

In some years with extremely high summer temperatures (between 30 and 40 °C) and no precipitation for a longer period of time, we observed mass mortality of mites in bee nests in the vicinity of Belgrade. In Japan heat treatment is a common practice to control *Ch. nipponicus* (Sekita and Yamada, 1993). The presence of mites in our populations in the following years was negligible. A succession of rainy summers without dry periods or extremely high temperatures favors mass development of hypopi in cells, with the result that the infestation in bee populations then increases significantly.

Chrysis ignita L. (Hymenoptera Chrysididae). The Chrysididae are distributed in the Palearctic and Nearctic, and the species *Ch. ignita* is encountered virtually all over Europe (Bouček, 1957). Adults have a metallic luster and body length of 5-10 mm. The head and thorax are coloured shiny blue-green, sometimes with a golden reflection. The abdomen is dark ruby-red. The underside of the abdomen is concave, which enables the insect to roll itself up into a defensive ball when threatened (Kimsey and Bohart, 1990).

These insects develop in the nests of bees and wasps. Their larvae feed on reserves of food gathered earlier by the host for its own progeny. They are often able to infest several successive cells in the nest of solitary bees. The prepupae of *Ch. ignita* spin an oval-shaped semi-transparent cocoon, in which they overwinter. The species *Ch. ignita* is sporadically registered in nests of *O. cornuta* and *O. rufa* in the vicinity of both Belgrade and Pisa.

Parasitoids

Monodontomerus obscurus Westwood (Hymenoptera Torymidae). This species is distributed in the north of Europe from the Moscow Province in Russia through Moldavia and the Caucasus to Central Asia (Nikolskaya, 1952; Bouček, 1977; Medvedev, 1978). It was introduced to North America, where it parasitizes species of the genera *Megachile* and *Osmia* (Hobbs and Krunić, 1971; Eves, 1970; Eves *et al*., 1980).

The adults are metallic blue-green in colour, with red eyes. The females have a thin and supple ovipositor. They are 3.8-4.9 mm long, while males are somewhat smaller and measure 2.4-3.0 mm in length (Stanisavljević, 1996) (figure 8). Adults emerge through the single uniform opening (the emergence opening, about 1 mm in diameter) on the bee cocoon that is in correspondence of a hole of the same diameter that these little wasps make in the surface of the nest (*Arundo* or *Phragmites* reed) (Felicioli, 2000) (figure 9a). The female perforates with its ovipositor the reed nest, and the cocoon and integument of the host larvae,

Figure 8. *M. obscurus*, adults.
(In colour at www.bulletinofinsectology.org)

Figure 9a. *M. obscurus*, *Phragmites* cane with the emergence openings.
(In colour at www.bulletinofinsectology.org)

Figure 9b. *M. obscurus*, overwintering prepupa in cocoons.
(In colour at www.bulletinofinsectology.org)

injects a paralyzing fluid, and then lays several eggs on or near the mature bee larva (Hobbs and Krunić, 1971; Stanisavljević, 1996). Larvae of the ecto-parasitoid feed on prepupae or white pupae of the bee. If an egg is laid in a cocoon with a dark pupa or adult bee, both the host and the parasitoid will die. Larvae of the parasitoid are white in colour, equipped with many bristles, and about 2.5 mm long upon reaching maturity (figure 9b). *M. obscurus* overwinters in the prepupal stage. Up to three generations of *M. obscurus* annually are possible in the vicinity of Belgrade (Krunić *et al*., 1999).

The parasitoid *M. obscurus* is frequently present in the nests of solitary bees. From 3 to 27 of parasitoids (10 on average) can develop in a single cell of *O. cornuta* (Stanisavljević, 1996). It easily penetrates paper tubes and massively parasitizes *O. cornuta* and *O. rufa*. We found *M. obscurus* specimens sporadically in bee nests in reeds, but did not find any in lamellar boxes in the vicinity of Belgrade. However, *M. obscurus* is found in significant numbers in both kinds of reeds (*Phragmites* and *Arundo*), but in very low numbers in lamellar boxes in the area of Pisa (Italy).

Melittobia acasta Walker (Hymenoptera Eulophidae). The species *M. acasta* spread from Europe to many other regions. It infests a large number of genera of bees, wasps, and some flies from Europe to Japan, North America, South America, and New Zealand (Medvedev, 1978; Dahms, 1984).

This wasp is often found in managed populations of solitary bees and bumblebees, to which it generally causes great damage due to its high reproductive potential, short life cycle, and significantly greater number of females (95%) than males (Dahms, 1984; Macfarlane and Donovan, 1989). The sexes of *M. acasta* differ in size, appearance of the head and wings, and colour. In addition to being larger and darker, the females have shorter knee-like antennae and larger functional wings. *M. acasta* overwinters as a prepupa in a cell of the host. Mating takes place inside the host cocoon, and only fertilized females emerge from the cocoon. There are several generations during the season, and the development cycle usually lasts about two weeks (Hobbs and Krunić, 1971).

In the case of *O. cornuta*, we found only a few cocoons infested with *M. acasta* over the last 20 years.

Leucospis dorsigera F. (Hymenoptera Leucospididae). This is a Palearctic species (Medvedev, 1978; Madl, 1989). Adult specimens are black with yellow markings on the body and legs. They measure 6-9 mm in length and are recognizable by their expanded and elongated hind legs (figure 10). Females of *L. dorsigera* lay eggs on a prepupa or pupa in the cocoon of the host. The larvae of this wasp usually do not spin a cocoon, but rather pupate in the cocoon of the host. Under European conditions, the species *L. dorsigera* most often has two generations a year. It was found sporadically in reeds with nests of *O. cornuta* and *O. rufa*, but did not cause any significant damage in populations of these bees.

Anthrax anthrax Schrank (Diptera Bombyliidae). This species belongs to the fauna of Western Europe. Body length is 10-13 mm. The body is black with an almost round head, the wings darkly sooty (figure 11). Characteristic tiny white husks are discernible on the abdomen. The females of this fly are attracted by the colour contrast created by a black disc on a different coloured background (Marston, 1964). When a female of *A. anthrax* hovers in front of the entrance of a solitary bee nest, it almost unerringly pitches an egg right into the nest with a flexure of the rear end of its body (Grandi, 1951). The first instar larva of this species is called planidium and is characterised by a high mobility so that it reaches the bee pollen provision before sealing occurs. It is still not clear if the planidium could pass through the mud partitions as described by Fabre (1937) or not. The planidium will not feed himself for a long time until the bee larva spins the cocoon. This is the right moment for the planidium to parasite the bee larvae. Once the bee has reached the pupal stage, the parasitization may occur. In this case it is possible to find the fragmented parts of the adult bee and the living fly larva together in the cocoon (figure 12).
This fly is present in almost all the Tuscany ecosystems (Italy) showing a parsivoltine and/or bivoltine cycle (Felicioli and Pinzauti, 1998), while it is univoltine in Spain (Fabre, 1937) and bivoltine in France (Du Merle, 1972).

Within some of the managed populations of both *O. cornuta* and *O. rufa* in Italy almost 50% of the sealed tunnels resulted parasitized by *A. anthrax*. The pupa of

Figure 10. *L. dorsigera*, adult.
(In colour at www.bulletinofinsectology.org)

Figure 11. *A. anthrax*, adult.
(In colour at www.bulletinofinsectology.org)

Figure 12. *A. anthrax*, larva feeding on *O. cornuta* pupa.
(In colour at www.bulletinofinsectology.org)

Figure 13. *A. anthrax*, "armed pupa" in *O. cornuta* cocoon.
(In colour at www.bulletinofinsectology.org)

this fly is characterised by a crest on the head so that it is defined as "armed pupa" (figure 13). The presence of the crest allows the pupa to open the cocoon and destroy the mud partitions of all the cells in order to reach the outside of the nest. Once the armed pupa has reached and perforated the last mud plug the fly will emerge leaving the host remains in its place. It is often possible to find hundreds of them already in the mud plug or on the floor near the *Osmia* nests.

The damage that this fly may cause is direct and/or indirect: the direct one is that each fly planidium kills the *Osmia* larva and it usually happens in the inner part of the nest containing the future female bees; the indirect damage is caused by armed pupae that start migration towards the outside of the nest during August (50% of the fly population). They crush all the cocoons containing bees still at the larval stage present in the nest, killing all of them and causing a very high mortality in the *Osmia* populations. In this case *A. anthrax* could be considered a nest destroyer (Felicioli, 2000).

In the area of Belgrade, the species *A. anthrax* is sporadically found in nests of *O. cornuta* and *O. rufa*. In Italy, on the other hand, this parasitoid is common, and in some localities it can parasitize up to 95% of the total number of cocoons (Felicioli, 2000). *Osmia* cocoons in lamellar boxes are much more frequently parasitized than cocoons in reeds, 47% and 5% respectively (Pratesi, 2004). *A. anthrax* is frequently present in populations of *O. rufa* in Germany, where it attains a degree of infestation of about 2% (Seidelmann, 1990).

The use of yellow painted lamellar boxes with only 0.5 cm deep black coloured holes induces *Anthrax* females to hover and spray their eggs in these "virtual" bee tunnels so that the combined use of these "*Anthrax* egg traps" and reed nests allows to highly reduce the percent of parasitization. Moreover, the imago flies usually emerge one week to fifteen days after the *Osmia* females, so that the practice of destroying the nests after the emergence of female bees is a useful method to reduce the fly parasitization (Pratesi, 2004).

Predators

Trichodes apiarius L. (Coleoptera Cleridae). The species *T. apiarius* is distributed all over Europe. Adults are often present on flowers of Compositae, where they feed on pollen and nectar. The body of adults is black-coloured, with conspicuously dark-red forewings. Males are 6.5-13 mm long, females up to 17 mm. Females lay their eggs in plant flowers or in various cracks, for example in cracks of lamellar boxes for solitary bees. The eggs are orangish-red and have the form of an elongated oval measuring on average about 2 mm in length. Larvae hatched from the eggs enter the nests of solitary bees and find eggs or larvae of the host, which they consume. After eating the content of one cell, they go on to the next cells, and consume their content as well. Larvae of *T. apiarius* at the first stages of development can be found in nests of *Osmia* at the end of June or beginning of July (Stanisavljević, 1996). They remain there until winter, when their development reaches the prepupal stage. The pinkish-red mature larva is 16-20 mm long and has a developed black head capsule (figure 14). The last larval stage spins a cocoon in spring and metamorphoses into a pupa in the course of about two weeks. Depending upon the external temperature conditions, emergence of adults lasts up to one month. The development cycle is completed in a year (Carré, 1980).

Figure 14. *T. apiarius*, larva.
(In colour at www.bulletinofinsectology.org)

We found *T. apiarius* in nests of *O. cornuta* and *O. rufa* in lamellar boxes and sporadically in reeds. It is most often the case that one larva destroys all the cells in a nest.

Insectivorous birds (*Parus major* L.; *Pica pica* L.; *Dendrocopus* spp; *Motacilla alba* L.) are frequent predators of orchard bees. During the period of activity of the bees, some birds feed on them, especially when there are not enough other active insects due to low temperatures. *O. cornuta* flies at relatively low temperatures, when it is an easy prey for birds. However, insectivorous birds can cause much greater damage to bee populations when they destroy their nests and extract cocoons before and during the period of hibernation. For this reason, nests must often be protected with chicken wire when the foraging activity of the bees ceases. The large concentration of bees at locations where they collect damp soil for their nests can also attract certain insectivorous birds, so that it is sometimes necessary to protect those places, too, with chicken wire.

For protection against birds during the foraging activity of orchard bees, shiny plastic strips or mirrors that move in the wind should be used to repel birds if it is unfeasible to install chicken wire.

Nest destroyers

Ptinus fur L. (Coleoptera Ptinidae). This species is widely distributed in the Holarctic (Bei-Bienko *et al*., 1949).

Adults are reddish-brown to black in colour, with yellow hairs. Each forewing has two white fields (figure 15a). The body of adults is 3-5 mm in length and about 3 mm wide. The antennae are long, often exceeding body length (Stanisavljević, 1996). Although they possess wings, specimens of this species do not fly. They are often present in the nests of many solitary bees. Females lay their eggs between the mass of pollen and the walls of the cell. Larvae are white and bent. The prepupa develops into pupa and later into overwintering adult. Some pupation chambers of *P. fur* are inside mud partitions (figure 15b), but several can be present together with the bee cocoon. Adults emerge from the cocoons at the beginning of the next spring. They have one generation a year. *P. fur* is often found in our bee populations, especially in used nesting material, where it feeds on waste of plant and animal origin. Under the conditions of south-eastern Europe, it does not cause significant damage to the bees.

Trogoderma glabrum Herbst (Coleoptera Dermestidae). This native member of the European fauna was introduced to North America and can therefore be found throughout the entire Holarctic (Eves *et al*., 1980).

Adults are oval-shaped and dark-black in colour. Virtually the entire surface of the forewings is overgrown with tiny black hairs. Three transverse bands of white hairs are especially conspicuous on the forewings. In older adults, the hairs drop off due to constant movement through the substrate, so that the surface of the forewings acquires a metallic black luster. Females measure 6-7 mm, while males are somewhat smaller. The females lay their eggs in nests of solitary bees and some other insects. The young larva is reddish-brown in colour and covered with long hairs. The mature larva attains a length of up to 6 mm. The life cycle of *T. glabrum* varies considerably as a function of temperature and availability of food reserves. The larvae are most often phytophagous, but it is not rare that this species also feeds on various kind of animal food, mainly parts of the bodies of dead insects. The species *T. glabrum* overwinters in all larval stages.

The presence of a large number of larvae of this species in different stages of development was observed in old nests of *O. cornuta* and *O. rufa* bees (figure 16). Under our conditions, it does not cause significant damage to the bees.

Figure 15a. *P. fur*, adult dorsal and ventral view.
(In colour at www.bulletinofinsectology.org)

Figure 15b. *P. fur*, cocoon in *Osmia* nest.
(In colour at www.bulletinofinsectology.org)

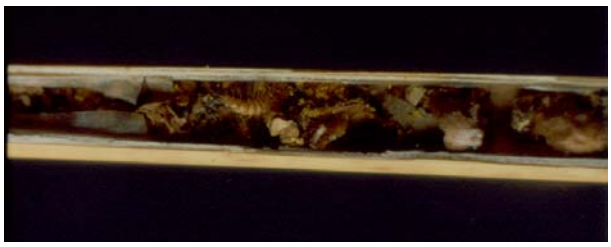

Figure 16. *T. glabrum* larvae in *Osmia* nest.
(In colour at www.bulletinofinsectology.org)

Plodia interpunctella Hübner (Lepidoptera Pyralidae). Larvae of *P. interpunctella* were registered in greater numbers in nests of *O. cornuta* and *O. rufa* in lamellar boxes than in reed bundles (Stanisavljević, 1996). They exerted no significant harmful influence in our bee populations.

Eumenid wasps: *Ancistrocerus parietum* L.; *Ancistrocerus* sp.; *Symmorphus* sp. (Hymenoptera Eumenidae). During the summer, these wasps enter empty reed bundles prepared for orchard bees and build cells in them in series (which they partition with damp soil, as bees do). They lay one single egg per cell and then provision it with larvae of Lepidoptera (Geometridae and Tortricidae) and Coleoptera (Curculionidae and Chrysomelidae). When the larva reaches the maturity, it spins a transparent cocoon. If the wasps overmultiply in the summer or face a shortage of empty reeds, they are capable of digging out sealed nests of *O. cornuta* and *O. rufa*. They withdraw the content of the bee nests and then build their own. Adults are active during the summer months, too. Many species of eumenids have only one generation throughout the year.

Cleptobionts

When females of *O. cornuta* and *O. rufa* bring the first loads of pollen and nectar to nests in multiplication shelters, they attract different cleptobionts, primarily ants, immediately upon installation of the nesting material. Attracted by the odour, they enter lamellar boxes and inhabited reeds. Several days after the onset of bee activity, a certain amount of pollen spilled by the bees (and not rarely even whole loads) can be found under the multiplication shelter and sometimes on reed bundles and in the reeds next to the openings. The ants massively collect pollen from the piles under shelters and from reeds, and carry it away to their own nests. Despite the frequent presence of ants around reeds and lamellar boxes during the period of bee activity, we did not observe them aggressively entering *Osmia* nests, and carrying away pollen and nectar. When the bees stop foraging, the abundance of ants in and around the shelter also declines. Although ants were sporadically seen in multiplication shelters all summer long, they were not observed to harm closed nests. If the soil under the shelter was sandy (which was the case at several of our localities), craters very soon appeared containing larvae of ant lions (*Euroleon nostras* Fourcroy), which hunted ants for food. It was not observed that the ant lions also hunted old female bees, which often crawled below the shelter and across their craters. In addition to collecting pollen, the ants also carried away dead male bees, which are often found under the shelter. In certain seasons when ants appeared in unusually great numbers, appropriate protective measures were taken (strips of sticky paper were set out or an insecticide was sprinkled around the supporting columns of the shelter). The sticky strips protected the bee nests not only from ants, but also from lizards (which are often seen among reeds in shelters) and from possible nocturnal visits by mice. The ants we observed (*Formica cunicularia* Latreille, *F. rufibarbis* F., *F. balcanina* Petrov & Collingwood, *Tetramorium caespitum* L., and *Camponotus fallax* Nylander) are cleptobionts, and their harmful influence on bees was negligible. Some species of ants in the vicinity of Belgrade can attack weak honeybee societies in an organized way, and "rob" and destroy them. For this reason, we employed preventive protective measures against ants on *Osmia* multiplication shelters.

Accidental nest residents

Osmia coerulescens L. This species is distributed in Southeast Europe and is a significant pollinator of Leguminosae. In the area of Belgrade, it has two generations a year. In the Pannonian Depression, it often inhabits lamellar boxes of *M. rotundata* in alfalfa fields. We found it very sporadically in narrow reeds and lamellar boxes set out for *O. cornuta* and *O. rufa*. It overwinters as a diapausing adult in transparent cocoons.

Liposcelis divinatorius Müller (Psocoptera Liposcelidae). Adults of the species *L. divinatorius* (the book louse) were found for the most part in old reeds with a lot of pulverized material in them. They did not cause any significant damage to the bees.

Lepisma saccharina L. (Thysanura Lepismatidae). This species was found sporadically in nests of *O. cornuta* and *O. rufa*.

Forficula auricularia L. (Dermaptera Forficulidae). Specimens of this species enter reeds and lamellar boxes in order to feed on organic waste in bee nests. They are often found in previously inhabited reeds, and frequently take refuge in empty reeds for the purpose of overwintering. They represent no significant threat in nests of populations of *O. cornuta* and *O. rufa*.

Vespid wasps: *Vespula germanica* L., *Polistes gallicus* L. and *P. bischoffi* Weyrauch (Hymenoptera Vespidae). In the fall, queens of these wasp species often enter empty reeds in bundles with orchard bee nests, where they spend the winter. They were not observed to dig up nests in order to overwinter in such places.

Xylocopa violacea L. (Hymenoptera Apidae). Specimens of *X. violacea* were frequently registered in the vicinity of multiplication shelters of *O. cornuta* and *O. rufa*, especially at the beginning of spring. From time to time, they entered large-diameter reeds, in which they sporadically established nests. Overwintering adults were sometimes found in large-diameter reeds in the fall.

The genus *Megachile*: *Megachile pilicrus* Morawitz, *Megachile* spp. (Hymenoptera Megachilidae). In Serbia and in Italy the bees of this genus usually appear at the end of spring, and are present all summer until the beginning of fall. They build nests of leaves, deposit pol-

len in them, and then lay an egg on that mass. The larvae spin a cocoon when they reach the prepupal stage and overwinter at that stage. These bees sporadically established their nests in our bundles for *O. cornuta* and *O. rufa* (figure 17).

The genus *Chalicodoma* (*Chalicodoma hungarica* Mocsary, *Chalicodoma* sp.) (Hymenoptera Megachilidae). Species of this genus build nests of fine sand or soil mixed with salivary gland secretions. From 7 to 10 cells in a row are found in one single nest. After drying, the cells are very firm and hard. The females bring nectar and pollen into the cell and lay an egg on this mass. Species of the genus *Chalicodoma* overwinter as prepupae, and adults appear at the beginning of the summer. They often build nests in empty reeds in bee shelters (figure 18).

Figure 17. Nest of the genus *Megachile* in *Phragmithes* cane.
(In colour at www.bulletinofinsectology.org)

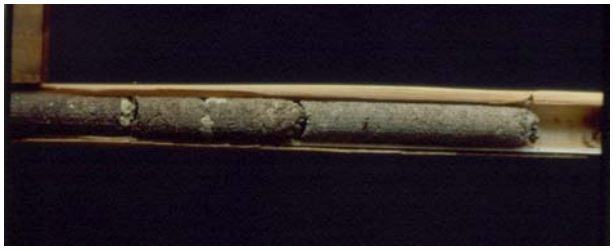

Figure 18. Nest of the genus *Chalicodoma* in *Phragmithes* cane.
(In colour at www.bulletinofinsectology.org)

Figure 19. Nest of the genus *Anthidium* in *Phragmithes* cane.
(In colour at www.bulletinofinsectology.org)

The genus *Anthidium*: *Anthidium florentinum* F., *A. melanurum* Klug (Hymenoptera Megachilidae). Species of this genus build nests in empty reeds for *O. cornuta* and *O. rufa* by first lining the cavity with moist wooly material collected from surrounding plants. They usually overwinter as prepupae or diapausing adults. Their nests were often found in reeds at all the localities where *O. cornuta* and *O. rufa* were reared (figure 19).

Conclusion

O. cornuta and *O. rufa* have a characteristic accompanying fauna in every region where they are reared for the pollination of orchards. With increasing abundance of the bees, the accompanying fauna increases in both diversity and abundance. Effective measures of protecting populations from the most harmful species of the accompanying fauna are being developed. If protective measures are taken, the accompanying fauna will not represent a limiting factor to the utilisation of these bees for orchard pollination.

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