Ecology of zoophilic flies in livestock biocenoses of Ukraine

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Introduction

Zoophilic flies are an ecologically plastic insect group adapted to the most diverse conditions of existence. The main criterion of designating this group was that larvae and imagoes of flies have ecological (trophic, topical, phoric) relationships with farm livestock and their vital products in the conditions of their indoor and pasture maintenance. Synanthropic fly species have biotic relationships with humans and their buildings, and consume the remains of organic products for their feeding and development. It is not easy to distinguish between synanthropic and zoophilic species since the existence of farm livestock is determined by human activity.

Flies cause significant economic damage. Hematophages that may vary the reducers of the animal industry are of particular importance. Synanthropic fly species are involved in the mechanical transmission of various pathogens, mainly bacteria (Soto et al., 2014; Khamesipour et al., 2020). Larvae of some species can develop under the skin of animals and cause the development of severe myiasis. The role of insects, including flies, as carriers of parasitic diseases is also important (Boyko et al., 2009; Soto et al., 2014; Khamesipour et al., 2020).

In modern animal husbandry of Ukraine, there are still some unresolved issues related to the high number of zoophilic flies in the territories of facilities and the spread of the animal infectious and parasitic diseases. A detailed study of the zoogeographical peculiarities of the dominant zoophilic fly species contributes to improving the effectiveness of measures to control ectoparasites. 27 zoophilic fly species have been identified in animal breeding complexes. The maximum number of parasitic Diptera species was recorded on cattle-keeping premises. The biological properties of Neomyia cornicina (Fabricius, 1781) (size, shape, colour, duration of preimaginal phase development) were studied. Also, we studied the dynamics of the number and daily activity of different fly species (Musca domestica Linnaeus, 1758, M. autumnalis De Geer, 1776, Stomoxys calcitrans (Linnaeus, 1758)). When studying the interspecies competition, a high-degree survival of M. domestica and M. autumnalis was determined in the conditions of critical nutritional deficiency (0.5 g of nutrient medium per larva) and increased density of individuals (the imago emergence was 38.6% and 34.0%, respectively). In similar maintenance conditions, the emergence of N. cornicina imago was low (14.6%). With a two-fold increase in the insectarium volume and in the amount of nutrient medium (2 g per larva), the imago emergence of M. domestica, M. autumnalis and N. cornicina increased to 64.0%, 39.2%, and 24.0%, respectively. With an even greater increase in the amount of nutrient medium (2 g per larva), the maximum emergence of imagoes of all the studied fly species was observed (M. domestica, M. autumnalis, and N. cornicina: 96.6%, 91.2% and 72.6%, respectively). In the conditions of interspecific competition, M. autumnalis suppressed N. cornicina even in conditions of a sufficient amount of nutrient substrate. In the competition between M. domestica and M. autumnalis, house fly dominated. Increasing the nutrient medium volume narrowed the gap between the competing species.

Keywords: zoophilic flies; species composition; Muscidae; cultivation; intraspecific; interspecific competition.

M. domestica is the dominant part of the zoophilic fly complex in terms of the species number. The species of this family are widely distributed in all zoogeographic regions (Muennworn et al., 2010; Ola-Fadunsin et al., 2020). Muscidae larvae live in various environments: decomposing plant remains, manure, compost, animal corpses, fungal fruit bodies, moss, silt, living tissues of plants and animals, etc. Most often, the larvae of muscid flies are facultative saprophages, or facultative, obligate predators. The larvae of some species act as ectoparasites or endoparasites that feed on blood or cause miasis (Lendzele et al., 2019). The type of muscids' nutritional structure is determined by the morphological features of their proboscis structure. Facultative hematophages and polyphages of Musca genus (including M. domestica) are characterized by loose-filtering-sucking type of the oral disk with developed preoral teeth and lamellar...
interdental plate. Flies that have this type of proboscis can damage fresh dried wounds of animals and absorb the released blood, lymph, intercellular fluid. Obligate hematophages – on the example of {imp}S. calcitrans{end} – have a glossinoid proboscis of the rasping-sucking type (Kulikova et al., 1999; Gregor et al., 2016). The reproductive strategy of muscids is also diverse. Saprophage species are characterized by three larval ages (trimorphic larvae). Larvae of age II or III (dimorphic or monomorphic larvae) emerge from the eggs of predatory muscid flies (Wall et al., 2008; Mavourenga et al., 2017).

There is competition between different fly species in nature, which manifests in behavioural characteristics during their feeding and reproduction. Especially fierce competition is observed among the inhabitants of temporary organic substrates such as corpses, excrement. The tense relationships between species over the evolutionary process have promoted the formation of highly sensitive sensory organs in imago flies which allow them to find food supplies as quickly as possible. For temporary living places, significant succession is characteristic, namely change of species complexes. If the species are adapted to life at the same successional stage, then the competition between them can be especially fierce. It is known that fly larvae that live on carrion or in animal excrement secrete specific substances that prevent the development of nematodes and fungi feeding on the same substrate. In other cases, female flies avoid egg-laying substrates stocked by competitive species and parasites; it was studied on the example of {imp}S. calcitrans{end} (Baleba et al., 2020).

The objective of the study was identifying the dominant species of zoophilic flies in livestock biocenoses of forest-steppe and steppe zones of Ukraine; to study the biology and ecology features of dominant fly species in field and laboratory conditions.

Materials and methods

Zoophilic flies were caught during active flight (May–October) in the premises for keeping cattle, pigs, sheep, poultry, on the territory of manure premises for cattle, pigs, sheep, poultry located in the territory of eight regions of Ukraine (Dnipropetrovsk, Donetsk, Zhytomyr, Zaporozhye, Kirovograd, Poltava, Kharkiv, Kherson oblasts) in the period from 2000 to 2020 (Table 1).

Entomological nets and exhausts were used to catch imago flies in livestock premises, as well as special traps recommended by a number of researchers (McCavy, 2018; Marchioro et al., 2020). Fly larvae and puparia were collected manually in places of their intensive breeding (rotting substrate, manure). Field collections on pastures were carried out according to the generally accepted methods used in entomology (Fasalati, 1971; Lamarre et al., 2018). Insect imagos were fixed in 70% ethanol solution for the further determining of the species composition. The collected fly larvae and pupae were incubated in insectarium for further laboratory cultivation. More than 9,194 imago and larvae specimens of zoophilic flies were collected during the research period. Assistance in identification of fly species was provided by I. A. Mashkey, A. A. Mishchenko, researchers of the Institute of Experimental and Clinical Veterinary Medicine.

After emergence from the puparia, imagos of house fly (M. domestica), face fly (M. autumnalis), and dung fly (Neomyia cornicina (Fabricius, 1781)) were selected for further laboratory cultivation. The flies were kept in cages measuring 50 × 50 × 50 cm. The frame of these containers was made of stainless wire covered with a piece of mesh fabric with the cell diameter of 1–2 mm. On the bottom of the cages, we placed dishes with a nutrient medium, water, and the substrate for laying eggs. A mixture of powdered milk with glucose (2:1) was used as the food for {imp}M. domestica{end} and {imp}N. cornicina{end} imagos. The daily consumption per specimen was 5–10 mg of the mixture. To feed {imp}M. autumnalis{end}, we used multicomponent nutrient medium consisting of milk powder, glucose, and stabilized bovine blood. Water was supplied as a moistened cotton swab in cups. Wheat bran steamed in hot water (1:2) was used for egg laying by {imp}M. domestica{end}. The substrate for eggs of {imp}N. cornicina{end}, {imp}M. autumnalis{end} was fresh cattle feces (450–600 g in a cage at the rate of 1.5–2.5 g per larva). 300–500 imagos of the same species were kept per one cage. In the boxes for insect cultivation, the temperature was maintained at 25–28 °C with 55–65% relative humidity and photoperiod of 16 hours. To quantify the laid eggs, the egg rafts of flies were placed in a 10 cm³ vessel that was filled with water. The upper layer of water was drained, and the settled eggs were counted (about 5000 eggs per 1 cm³). Then water was added up to the mark of 5 cm³, and the resulting suspension was shaken and, without letting it settle, 1 cm³ (about 1000 eggs) was collected. Then, the fly eggs were placed in 0.5 L containers with a cultivation medium.

For the experiments on the intraspecific competition of three cultivated Muscidae species in the conditions of nutrient substrate deficiency, we used 300 and 500 cm³ containers. The species were kept separately. Samples of cattle feces (25, 50, 100 g) were added to the cages and 50 first-age larvae were placed in each. We used two fly species in each of the experiment on interspecific competition in the conditions of nutrient substrate deficiency. In the first experiment, {imp}M. autumnalis{end} and {imp}N. cornicina{end} were used, and {imp}M. domestica{end} and {imp}M. autumnalis{end} in the second. We used 500 cm³ containers with weighed amounts of feces equaling 25, 50, 100 g. A total of 50 first-age larvae of two fly species were placed in each cage with a certain amount of cow feces.

Results

Twenty seven species of zoophilic flies were identified in the specialized livestock complexes for cattle, pigs, sheep, poultry located in the territory of eight regions of Ukraine (Table 1). Among them, Muscidae, Calliphoridae, Fanniidae, and Sarcophagidae accounted for 74.1%, 14.8%, 7.4%, and 3.7%, respectively. The largest number of zoophilic fly species was recorded in the premises for cattle keeping. The lowest number of parasitic Diptera species was recorded in poultry farming premises. {imp}M. domestica{end} and {imp}S. calcitrans{end} were identified in all livestock and poultry complexes (Table 2).

### Table 1

| Sampling area | Number of samples, pcs. | Date | Number of samples, pcs. | Date |
|---------------|------------------------|------|------------------------|------|
| Dnipropetrovsk oblast, Verkhnedniprovsky district, Dniprovsky urban-type settlement | 48°55′46″ N 34°25′11″ E | 2009 | June | 20 |
| Donetsk oblast, Slavyansky district, Alexandrova village | 48°49′11″ N 37°22′55″ E | 2010 | June | 20 |
| Donetsk oblast, Slavyansky district, Dmitrovka village | 48°51′15″ N 37°22′55″ E | 2011 | September | 20 |
| Zhytomyr oblast, Popelnyansky district, Matarovka village | 49°59′51″ N 38°32′24″ E | 2007 | June | 20 |
| Zhytomyr oblast, Popelnyansky district, Novoselota village | 49°54′10″ N 39°37′37″ E | 2008 | July | 20 |
| Zhytomyr oblast, Popelnyansky district, Erdik village | 49°39′56″ N 29°34′42″ E | 2009 | June | 20 |
| Zhytomyr oblast, Popelnyansky district, Sokolova village | 49°59′16″ N 29°19′57″ E | 2010 | June | 20 |
| Zhytomyr oblast, Baranovsky district, Ostrozhok village | 50°23′44″ N 27°44′22″ E | 2011 | July | 20 |
| Donetsk oblast, Slavyansky district, Alexandrova village | 48°49′11″ N 37°22′55″ E | 2012 | – | – |
| Donetsk oblast, Slavyansky district, Dmitrovka village | 48°51′15″ N 37°22′55″ E | 2013 | – | – |
Zoophilic fly species composition in livestock biocenoses of forest-steppe and steppe zones of Ukraine

| Sampling area | Date | Month | Premise type | Spike camp | Pasture |
|---------------|------|-------|--------------|------------|---------|
| Zhytomyr oblast, Buranovsky district, Rogachev village | 2020 | August | – | – | 20 |
| 50°24′36″ N 30°34′32″ E | 2013 | July | 20 | – | – |
| 48°18′20″ N 35°34′20″ E | 2001 | June | 20 | 20 | – |
| Kyivograd oblast, Petrovsky district, Luganka village | 2002 | June | 20 | 20 | – |
| 50°20′40″ N 35°34′35″ E | 2003 | August | 20 | – | – |
| Poltava oblast, Novosanzharsky district, Kuntsevo village | 2005 | July | 20 | 20 | 20 |
| 49°22′30″ N 34°23′30″ E | 2011 | September | 20 | – | – |
| Poltava oblast, Novosanzharsky district, Novi Sanzhary urban-type settlement | 2000 | June | 20 | 20 | 20 |
| 49°20′30″ N 34°18′45″ E | 2013 | August | 20 | 20 | 20 |
| Poltava oblast, Kotelevsky district, Kotela village | 2004 | May | 20 | 20 | 20 |
| 50°47′30″ N 34°45′30″ E | 2012 | July | 15 | 15 | – |
| Poltava oblast, Gadyatsky district, Veprik village | 2014 | September | 20 | 20 | – |
| 50°30′30″ N 34°10′30″ E | 2012 | June | 20 | – | – |
| Kharkiv oblast, Zmiev district, Zadonetsky village | 2002 | June | – | – | 20 |
| 49°38′40″ N 36°21′55″ E | 2003 | July | – | – | 20 |
| 2010 | June | – | – | 20 |
| 2006 | July | – | – | 20 |
| Kharkiv oblast, Dergachev district, Malaya Danilovka village | 2009 | August | – | – | 20 |
| 50°41′30″ N 36°9′46″ E | 2010 | July | – | – | 20 |
| 2019 | July | – | – | 20 |
| 2020 | August | – | – | 20 |
| 2006 | May | – | – | 20 |
| 2007 | June | – | – | 20 |
| Kharkiv oblast, Kharkiv district, Temnovka village | 2008 | June | – | – | 20 |
| 49°47′10″ N 36°20′29″ E | 2009 | August | – | – | 20 |
| 2010 | June | – | – | 20 |
| 2016 | August | – | – | 20 |
| 2018 | June | – | – | 15 |
| Kharkiv oblast, Krasnogvar district, Martynovka village | 2010 | July | 20 | 20 | – |
| 49°18′50″ N 35°24′50″ E | 2015 | June | 20 | 20 | – |
| Kharkiv oblast, Novomolodachsky district, Sonovka village | 2011 | June | 20 | – | – |
| 49°25′24″ N 35°39′20″ E | 2012 | August | 20 | – | – |
| Kharkiv oblast, Zmiev district, Pervomayskoye village | 2008 | June | 20 | 20 | – |
| 49°29′10″ N 36°19′20″ E | 2009 | June | 20 | 20 | – |
| 2017 | June | 10 | 10 | – |
| Kherson oblast, Kakhanovsky district, Tavrichanka village | 2012 | July | 20 | 20 | – |
| 46°33′10″ N 33°17′9″ E | 2013 | July | 20 | 20 | – |

Table 2
Zoophilic fly species composition in livestock biocenoses of forest-steppe and steppe zones of Ukraine

| Family name | Species name | Animal husbandry premises for housing of: |
|-------------|-------------|------------------------------------------|
| Fanniidae   | *Fannia canicularis* (Linnaeus, 1761) | cattle | + | + | – | ++ |
| *E. scalaris* (Fabricius, 1794) | + | + | – | – | – |
| *Haematobia irritans* (Linnaeus, 1758) | + | – | – | – | – |
| *H. stillans* (Bezzi, 1907) | ++ | – | – | – | – |
| *Haematobia atra* (Bezzi, 1895) | + | – | – | – | – |
| *H. stimulans* (Meigen, 1824) | + | – | – | – | – |
| *Hydrotaea dentipes* (Fabricius, 1805) | + | – | – | – | – |
| *Mesembrina meridiana* (Linnaeus, 1758) | + | – | – | – | – |
| Muscidae    | *Morellia hortorum* (Fallada, 1817) | ++ | – | – | – | – |
| *M. simplex* (Loew, 1857) | + | – | – | – | – |
| *Musca autumnalis* De Geer,1776 | ++ | – | – | – | – |
| *M. domestica* Linnaeus, 1758 | + | – | – | – | – |
| *M. karschii* Porchinsky, 1910 | + | – | – | – | – |
| *M. osiris* Wiedemann, 1830 | + | – | – | – | – |
The study revealed that the female *N. cornicina* lays 30–47 eggs per raft, assembled by a special secretion into 1–2 groups comprising 15–30 specimens. The dung fly looks for a recess in the thickness of the cowpat and lays eggs at 1.5–2.5 cm depth from the surface of the substrate. Egg laying was observed once every two days. Females lay eggs throughout their life. The eggs are white or yellowish-white, elongated in shape, slightly narrowed towards one end. On the dorsal side, the eggs are convex; on the ventral side are straight or concave with two ribs. The length of the eggs is 1.9–2.0 mm, the width in the center equals 0.15–0.16 mm. After the larva emerges, the rupture line on the shell of the nucleus passes from the expanded to the narrowed end. First-age larvae were colourless, light grey, and gradually turn into blue-green as the cuticle dries. The sex of the fly from the pupa lasts up to 3 hours. After the emergence, imagoes are active in winter, no breeding sites of *S. calcitrans* were found in live-stock premises. In December and January, number of imagoes in the premises was low. The fly index was 1–3 specimens per animal. Since this species prefers manure mixed with vegetation for laying eggs, it is possible that individuals of the autumn generation are active in winter.

The study revealed that under conditions of intraspecific competition, the species of the *Musca* genus were characterized by high degree of survival in conditions of critical nutritional deficiency and increased density of individuals (50 larvae per 25 g of nutrient medium with an insectarium volume of 300 cm³). The low level of emergence of *N. cornicina* imagoes (14.6%) indicates that the larvae do not accumulate enough nutrients for their metamorphosis (Table 3).
of adults from puparia increased: *N. cornicina* by 24.0%, *M. autumnalis* by 28.6%. In the optimal conditions of maintenance (2 g of feces per larva), there was uneven increase in the number of imagoes that emerged between the two species. In the conditions of interspecific competition, *M. autumnalis* suppressed *N. cornicina*. This was especially evident in the presence of sufficient amount of nutrient substrate (100 g of feces). Thus, emergence of *M. autumnalis* images was close to 100%, and the emergence of *N. cornicina* adult individuals was two times lower (Table 4).

When dung flies were cultivated in monoculture, the indicators of imagos emerging from puparia (in similar keeping conditions) were almost 1.5 times higher (Table 3).

### Table 4

| Weight of feces, g | Species name | Pupated larvae, ind. | Imago emerged, ind. | Survival rate of individuals, % |
|-------------------|--------------|----------------------|---------------------|--------------------------------|
| 25                | *N. cornicina* | 8.3 ± 2.5            | 3.0 ± 1.0           | 60.0                           |
| 50                | *M. domestica* | 133.3 ± 3.1          | 7.3 ± 1.5           | 14.6                           |
| 100               | *N. cornicina* | 180.0 ± 2.0          | 150.3 ± 3.6         | 30.0                           |
| 100               | *M. domestica* | 236.3 ± 3.2          | 216 ± 4.0           | 43.2                           |
| 25                | *M. autumnalis* | 293.3 ± 3.5         | 253.3 ± 5.6         | 50.6                           |
| 50                | *M. domestica* | 493.3 ± 5.0          | 483.3 ± 5.1         | 96.6                           |
| 100               | *M. autumnalis* | 293.3 ± 3.0        | 280.4 ± 6.6         | 56.0                           |
| 100               | *M. domestica* | 130.0 ± 3.0          | 73.2 ± 2.5          | 14.6                           |
| 50                | *M. autumnalis* | 426.4 ± 6.4        | 413.4 ± 4.2         | 82.6                           |
| 100               | *M. domestica* | 233.3 ± 3.8          | 210.6 ± 4.6         | 42.0                           |
| 100               | *M. domestica* | 400.0 ± 6.2          | 486.3 ± 4.2         | 97.2                           |
| 100               | *M. autumnalis* | 453.4 ± 4.0         | 430.4 ± 4.0         | 86.0                           |

During the interspecific competition between *M. domestica* and *M. autumnalis*, the house fly took the leading position. A high percentage of both the emerging pupae and imagos of this species was recorded: more than half of the individuals participating in the experiment, even with a maximum nutritional deficiency (25 g feces weight). In similar conditions, the emergence of adult *M. autumnalis* was three times lower. Increase in the amount of nutrient substrate increased the survival rate of individuals of both species. The presence of nutrient medium in insectariums in the amount of 50 g reduced the gap between competing species; the imago emergence was 2:1 in favour of *M. domestica*. When the use of 100 g of feces (at the rate of 2 g per 1 larva), high rates of larvae pupation and imago emergence of both studied species were recorded. The gap between the species further reduced, and *M. domestica* individuals slightly dominated (Table 4).

### Discussion

In biocenoses, populations of different species are in continuous interaction. Competitive relations in the process of long-term interspecific competition are an important factor regulating the abundance and composition of species in a community. Ecological niches of species often overlap, which creates conditions for interspecific competition. Competition arises if the resource is limited, and species have similar needs, for example, they use the same food and habitat for their offspring development (Kishi, 2015). In our laboratory experiments determining the competitiveness of the species, we used *N. cornicina*, *M. domestica*, and *M. autumnalis* which require manure as the resource for their development in preimaginal phases. Of these three fly species, in conditions of increased density of both the emerging pupae and imagoes of this species was recorded: more than half of the individuals participating in the experiment, even with critical shortage of a nutrient substrate (0.5 g of feces per larva) and increased density of individuals. For *N. cornicina* imagos, very low emergence was recorded (14.6%). After 2-fold increase in the volume of insectarium and weight of feces (1 g per larva), the emergence of *M. domestica*, *M. autumnalis* and *N. cornicina* imagos increased by 25.4%, 5.2%, and 9.4%, respectively. We observed the highest rate of emergence of imagos of all the studied fly species with increase in the amount of nutrient medium to 2 g per larva.

The study revealed that during the intense flight period (June–July), the number of *M. domestica* imagos simultaneously attacking one animal was 500–600 individuals. We should note that *M. autumnalis* imagos attacked cattle only in the first half of the day. One animal can be attacked simultaneously by 80–100 individuals. The seasonal dynamics of *S. calcitrans* abundance were characterized by two peaks. The first one was in late June – early July (30–50 individuals per animal). The second one occurred in September (50–100 individuals per animal). Mass attacks of *S. calcitrans* on cattle were observed at lunchtime. In the evening hours, the flies were low-active.

As for the competitiveness of the three fly species, the study revealed that the species in *Muscidae* genus were characterized by high-degree survival in the conditions of interspecific competition due to critical shortage of a nutrient substrate (0.5 g of feces per larva) and increased density of individuals. For *N. cornicina* imagos, very low emergence was recorded (14.6%). After 2-fold increase in the volume of insectarium and weight of feces (1 g per larva), the emergence of *M. domestica*, *M. autumnalis* and *N. cornicina* imagos increased by 25.4%, 5.2%, and 9.4%, respectively. We observed the highest rate of emergence of imagos of all the studied fly species with increase in the amount of nutrient medium to 2 g per larva.
(56.0%). In similar conditions, the emergence of adult M. autumnalis was three times lower. Increase in the nutrient medium (50 g weight of feces) narrowed the gap between these competing species. With the use of 100 g of feces, we observed high rates of pupation of larvae and emergence of imagos of both species. Individuals of M. domestica slightly dominated.

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