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MÁTÉ ZSOLT OTÁRTICS

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KAPOSVÁR CAMPUS

Institute for Wildlife Management and Nature Conservation

Head of the doctoral school:
PROF. DR. ANDRÁS SZABÓ
Doctor of the Hungarian Academy of Sciences

Supervisor:
DR. HABIL. SÁNDOR FARKAS, associate professor

ANALYSIS OF HORSEFLY COMMUNITIES AND INVESTIGATION OF THEIR DISTURBANCE IN HORSES

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Written by:

MÁTÉ ZSOLT OTÁRTICS

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RESEARCH BACKGROUND

Horseflies possess great economical and ecological impact on our agriculture as there are over 4,500 species on Earth, 61 of which has been detected in Hungary. Most species need blood to lay eggs. These species suck blood not only from many of our farm animals, but also from humans. The pain associated with bite impairs the welfare of the animal and if the bloodsuckers attack in a mass, they may go wild even resulting an accident. In areas where there are many horseflies, the daily blood loss can reach 300 ml per animal, which may have a negative impact on cattle milk and meat production, or the horse competition performance.

Horseflies may also contribute to the spread of pathogens, which makes them not only unpleasant but also dangerous. They have been shown to spread the pathogens of tularemia, anthrax, epidemic anemia, swine cholera, and foot-and-mouth disease. Horseflies are also important from a human, animal health and economic point of view. In order to defend against them, it is necessary to know the common and frequent species, their spatial and temporal pattern, their biology, and their possibilities of trapping. To determine the economic impact caused by horseflies, their effects on animals must be examined. The results of research on seasonal and diurnal activity may be particularly important for farms as well as equestrian barns where education and horse riding tours are organized.

One of the most effective methods of protection against horseflies is mass collection with traps. Several studies have been published in the last decade analyzing the effect of polarized light on insects. Studies have shown that the horseflies are also attracted to horizontally polarized light-reflecting surfaces. The discovery of the phenomenon was an important step in the control of horseflies, because using traps operating on this principle (H-trap), horseflies can be massively reduced with high selectivity. The efficiency and selectivity of the new trap have been scientifically examined by some studies. It is not known what the relationship is between the size of the area to be protected and the number of traps to be placed, what other insects it attracts, how dangerous they are for insect species of conservation value. There is also no information on the optimal placement of traps, although this is probably one of the most important factors influencing the effectiveness of traps.

In riding halls, it is a common behavior of horses for protecting themselves from horseflies by moving their heads, their legs and their tails, or possibly going into the shade. These behaviors can be counted, and the numbers can be used to estimate the extent of the negative impact of the horseflies.

OBJECTIVES OF THE STUDY IN THIS DISSERTATION

I. Faunistic and ecofaunistic survey: identification of abundant (occurring in many places) and mass (high specimen) horseflies species, based on the analysis of material from mass collections in several sampling areas several times a year. Collection of horsefly samples in previously unexplored areas of Somogy county. Comparison of our results with previous studies for detecting long term trends in their density.

II. Analysis of daily activity pattern of horseflies: examination of the diurnal activity pattern of common and abundant horsefly species by sampling them at four different times per day.

III. Investigation of the seasonal activity pattern of horseflies from the beginning of swarming (May) to the end of swarming (September) with continuously operated traps.

IV. Prognostic survey: examination of the factors influencing the catch rate of the H-trap; the effect of direct sunshine and the reflectance of the lure ball on the efficiency of the trap.

V. Ethological survey on horses: qualitative-quantitative analysis of the daily and seasonal activity of horseflies and the related anti-bite behavior of horses, based on parallel video recordings of horse behavior and H-trap collections on equestrian farms.

MATERIAL AND METHODS

We collected the horseflies for faunistic and community structure analyses at 18 sampling sites from 2017 to 2019, between May and September. The collection sites are located in Southern Transdanubia: Csokonyavisonta, Darány, Drávaszentes, Drávatamási, Homokszentgyörgy, Juta, Kálmáncsa, Lábod, Lad, Somogytarnóca, Szulok, Toponár and Zselickisfalud. As part of the 2019 Biodiversity Days, we also collected horsefly samples at the environs of Apaj and Dömsöd. Investigations of the effectivity of the traps, experiments on diurnal and seasonal activity of flies, and horse ethological studies were performed at the Vital Equestrian Club of Taszár, at the Nyargalók Equestrian Club of Sántos, and at the Ropoly site belonging to the MATE Kaposvár Campus.

For the collection of the insects we used the so-called H-trap, a special version of the “canopy-trap”, which is made of white funnel-shaped net, a shiny black lure ball and a collecting box. The trap is attached to an L shaped support structure. Among the factors influencing the trap operation, the effect of placement was examined in Sántos, where the catch success of traps placed at either sunny or shady spots in the stables were compared, and then the experiment was repeated after rearranging the traps several times. In Ropoly, we compared the catch results of traps equipped with either shiny or matte lure balls.

During the behavioral data collection, the frequency of anti-horsefly behaviors of the horses (tail strokes, head and neck shaking, head disturbance, ear twitch, foot disturbance, skin movement) were observed from video recordings. We compared data collected at four times of the day (9.00, 12.00, 15.00 and 18.00), when we took 5-5 minute recordings of the same ten horses and then counted the frequency of the above listed behaviors.

The specimens were systematically arranged in collection boxes. For species identification we used the keys developed by CHVÁLA et al. (1972), MAJER (1987b) and KRČMAR et al. (2011).

In a comparative analysis of the study years, the relative frequency and frequency (what percentage of the samples occur in a given species) were determined.

Frequency was also analyzed by calculating ISA (Index of Species Abundance) values because this index takes into account relative frequency and frequency at the same time.

Diurnal and seasonal data were analyzed by Tukey and Kruskal-Wallis post-hoc tests. In the analysis of diurnal activity, the number of horseflies trapped per time of day was used as a variable and their averages were compared.

The analysis of seasonal activity was based on the grouping of collection data by month and decade (10-day period).

In the prognostic analysis section, the homogeneity of the data was checked by chi-square test. The operation of traps installed in different lighting conditions and with different colored balls was compared using Tukey's test.

In the ethological study, statistical analysis of video recordings of horses was performed using Tukey and Kruskal-Wallis tests.

For statistical analyses we used MS Office 2016 Excel and GraphPad InStat 3.05 software package.

RESULT AND DISCUSSION

Faunistics and community structure

During the trapping sessions, altogether 31,980 horseflies belonging to 34 species were collected. The most common species were Haematopota italica, Haematopota pluvialis, Tabanus bromius, Tabanus autumnalis, Tabanus tergestinus and Tabanus sudeticus, which were found at most sampling sites. In all collected materials, according to their relative frequency, the most common species were H. italica (0.32), T. tergestinus (0.18), T. bromius (0.15), T. autumnalis (0.8), T. sudeticus (0.7) and H. pluvialis (0.05). Individuals from these six species accounted for 85% of the total material collected.

Species classified as rare in Hungary have also been found: Haematopota bigoti, Hybomitra acuminata, Hybomitra distinguenda, Hybomitra ukrainica, Phylipomyia graeca, Sylvinus alpinus, Tabanus quatornotatus, Therioplectes gigas and Haematopota ocelligera. H. ocelligera has previously been described from the Mecsek under the synonymous name H. hispanica, but none of the names is on the official Hungarian species list, so it can be considered a new species for fauna.

Diurnal and seasonal activity pattern

In Sántos, in the morning, the number of horseflies was significantly lower than at noon, early and late afternoon. The number of horseflies was highest in the early afternoon hours, about double the time between 9-12 and 15-18 hours. The difference was not significant between the numbers of noon, early, and late afternoon hours. Thus, based on the catch results, the horseflies will exert a strong load on the horses from 12 noon.

In our Ropoly study, the lowest activity was also observed in the early morning sample, which was significantly different from all the others. By number, the mean of the sample taken at noon was the highest, at which the 15-hour mean was significantly lower. At 18-hour we again found a high value, which was almost identical to the 12-hour sample and was significantly higher than the 15-hour sample. In the morning hours, the average was significantly lower on both days compared to the rest of the day. From 9 a.m., activity increased and was highest numerically in the sample taken at 3 p.m. on both days, but there was no longer a statistical difference between the samples collected at 12 p.m., 3 p.m., and 6 p.m.

For the analysis of seasonal activity, we used the data of traps operated in Sántos in 2018. Using the seasonal intervals recommended by Hayakawa, spring species were *T. bovinus*, *H. crassicornis, T. autumnalis, T. maculicornis, H. subcyllindrica, Hy. ciureai, Hy. muehlfeldi* and *Hy. bimaculata,* a summer species were T. *tergestinus, H. italica, T. bromius, T. sudeticus, H. pluvialis* and *A. loewianus*, and finally an autumn species were *He. pellucens* and *T. glaucopis.*

According to the results of the flight period analysis, the horseflies appear in the beginning of May and disappear in the first half of September. The seasonal cycle of each species is different, some species appear in the beginning of the season, in May, others begin to fly in the second half of the summer. *H. italica, H. pluvialis, H. subcyllindrica, He. pellucens, Hy. ciureai, T. autumnalis, T. bromius, T. sudeticus,* and *T. tergestinus*, the longest flight period of five months was observed, which lasted from May to September. Further nine species were collected at the beginning of the season, in May, but the active period of *H. ocelligera, T. bovinus* and *T. spodopterus* ended in July, for other species (*Ch. caecutiens, H. crassicornis, Hy. bimaculata, Hy. muehfeldi, T. maculicornis*) ended in August. Some species, such as *Hy. distinguenda, Ph. graeca, S. alpinus* and *Th. gigas* only appeared in June. The *Ch. viduatus, Hy. ukrainica, S. alpinus, T. cordiger, T. paradoxus,* and *T.*

spodopterus were absent from the samples in both May and September, so we could only collect them in the summer months.

The effect of trap placement on efficiency

Based on our initial results, most of the traps that collected specimens were always located in open, sunny areas, so we hypothesized that the operation of the traps could be affected by lighting. In testing this hypothesis, we installed traps in a shady and sunny location in the middle of the farm. Analyzing the data, we found that sunny traps caught significantly more horseflies than shady traps. These results suggest that H-traps work best in sunny, open areas, where they can catch 30 to 40 times more horseflies than those in shady locations. Thus, the placement of H-traps greatly affects their efficiency.

Effect of lure ball surface on trap efficiency

There was no statistical difference between the shiny and matte traps, taking into account the catch throughout the day. When comparing the times of day, we did not find any difference between the traps either. Based on the analysis of the data, the most abundant species were *T. bromius*, *T. tergestinus*, *T. sudeticus*, and *H. italica*. A *T. sudeticus* egész napos összesítésben mind a matt, mind a fényes csapdát egyformán preferálta, napszakos bontásban azonban a 18.00 órai mintában, a matt csapdákban szignifikánsan több volt belőle. Hasonlóan a *T. tergestinus* esetében sem volt különbség a két csapdatípus között az egész napos összesítésben. *T. bromius* showed no difference in its preference between the two trap types either in daily aggregation or during the day. In the case of *H. italica*, as in the previous species, no significant difference was observed between shiny and matte traps.

The effect of horseflies on the behavior of horses

Horse behavior in Taszár reflected the presence of horseflies in 2019, and changed in parallel to horsefly densities, as the number of tail strokes proved to be high in both July and August, while in the last third of September it proved to be significantly lower compared to the summer months. Similarly, the average head shake was significantly lower in September compared to the summer months, while there was no significant difference among the latter samples.

In a comparison by time of day, we found that the number of tail strokes in the morning hours was significantly lower than in the afternoon. The average head-shaking movements in the afternoon were well above those in the morning and early evening. It is also interesting to see the number of movements of the foot: in the morning and early evening hours, this behavior was noticeably, significantly less often, than in the period from noon to evening time.

We saw a change in behavior patterns in September. By this time, the horseflies will disappear, but many other insects are still trying to get food around the horses (flies, mosquitoes). As a result, the number of defensive movements is significantly reduced. The average of tail strikes in September was significantly lower than in July, except in the early evening hours, which can be explained by the evening activity of mosquitoes. Even more spectacularly, in all four periods of the day studied, the number of head-shaking movements that the horse applies specifically to the horseflies decreased in September.

The self defending foot movements by the horses also became noticeably lower in September than it was in July. In this case, in the period from noon to 6 pm, the average decreased to 14–21% of the July value, when the horseflies were most active in summer, while in the morning and early evening, the decrease was not as large.

In the Sántos Equestrian Club, the rough comparison of summer and autumn samples was the same as in Taszár: the average number of tail strokes and head-shaking movements per minute was significantly higher in summer than in autumn sampling. Comparing the frequency of defensive movements at different times of the day, we obtained partly similar and partly different results to those observed in Taszár. There was no difference between the mean of the tail strokes in the morning and evening and in the early and late afternoon samples, but the latter differed significantly from the morning and evening samples. The intensity of head and foot disturbing movements did not show diurnal dynamics, no significant difference was observed between the means. This is a significant difference from the Taszár data. Seasonal comparisons of time of day showed significant differences. The average number of tail strokes in October at each time of day was only a fraction of the June value. The number of head-disturbing movements decreased even more drastically, while no foot-disturbing movement was observed at all in October. The drumming movements followed the usual summer pattern in June, so their average was the lowest in the morning and the highest from noon. In October, only one horse pounded only once. Thus, the seasonal comparison clearly showed that horses perform significantly more horseflies-repelling movements in summer than in the autumn months.

The activity of horseflies can be inferred from the number of insect individuals collected at different times of the day. We found a strong correlation between these numbers of individuals and the daytime averages of the defensive movements of horses. In particular, horses 'tail bumps, skin tremors, and head-disturbing movements were closely related to the number of traps that were trapped.

No correlation was found between ear twitching and horsflies mass. In the analysis of the relationship between the two most common horsfly species and the three most commonly used behaviors, we found a very close relationship between equine behavior and the presence of *T. tergestinus*, while the number of tail strokes and head disturbances is closely related to *T. bromius.*

CONCLUSIONS AND RECOMMENDATIONS

Based on the results of faunistic research, it is possible to classify some of the 61 species of horseflies that can be categorized as dangerous for outdoor animal husbandry. Small numbers of species are economically insignificant, while common, mass species can cause serious problems. Faunistic studies reveal which species' way of life, reproduction, development, feeding habits, behavior and biology need to be researched more thoroughly in order to develop an appropriate control method against them. Summarizing the frequency and mass data as well as the ISA index, we found that the same six species are at the forefront in both respects: *H. italica, H. pluvialis, T. autumnalis, T. bromius, T. sudeticus* and *T. tergestinus.* So, these are common and mass species, potentially the most serious problem in outdoor animal husbandry. Research into these species should focus in the future.

Examining the seasonal activity of horseflies can be important to farmers in several ways. Knowing the data, they can plan the timing and intensity of the defense. When scheduling the use of pastures or equestrian tourism businesses, it is also important to know which mass of horsefly species and in what period they are flying. Based on our results, the most critical period is between June 20 and August 10, when the largest numbers of mass and common species fly. Knowledge of the diurnal activity of horseflies can also be important e.g. in summer, when planning the timing of outdoor equestrian competitions or equestrian tours. The activity of the horseflies is usually low in the morning, then increases with increasing temperature, and finally becomes lower again in the late afternoon and early evening. A different, but not yet known pattern of activity has also been found in the forested sample area, where the activity of the horseflies is high at noon and in the evening, and low at noon.

One of the main goals of our research was to increase the effectiveness of horsefly control methods. In this context, we tested how to optimize the conditions for the operation of the H-traps, which in practice means that the trap should catch as many horseflies as possible. In our experience, although many companies are already selling these traps, there are still many questions, or false unsubstantiated claims about their operation. We found that traps installed in a shady place practically do not work, whereas traps placed in a sunny area catch hundreds of individuals per day. This is an important information to disseminate because riding stables buy traps in vain if they do not work because they are often hung on trees where they are in the shade.

In the analysis of defensive behaviors by the horses we obtained clear indications that certain behavioral elements are performed to reduce fly landing and prevent the painful bitings. The diurnal frequency of several sets of movements (tossing, head, foot disturbance, tail flapping) is strongly correlated with the daily activity pattern of the horseflies, which was observed in both the Sántos and Taszár riding stables. We also found significant differences between the summer and autumn frequencies of the same behaviors.

NEW SCIENTIFIC RESULTS

1. I collected over 27 000 horsefly in the trapping sessions and obtained new faunistic data on horsefly presence in Hungary, enabling us to know the Hungarian horsefly fauna and the distribution of the species better.
2. These trapping sessions performed at several locations provided extensive data on the structure and temporal changes of Hungarian horsefly communities throughout the season
3. I identified a set of common and abundant horsefly species that can be classified as dangerous for animal husbandry, which are *H. italica, H. pluvialis, T. autumnalis, T. bromius, T. sudeticus* and *T. tergestinus*.
4. I detected a new species for the Hungarian horsefly fauna, the *Haematopota ocelligera*.
5. By experimentally manipulating the spatial position of H-traps, I have proved that trapping success is restricted to sunny places, and that result can easily applied in practice as a recommendation that traps should be installed in a place where they are not in the shade.
6. I described the defensive behavior of horses and demonstrated that the frequency of defensive behaviors by horses reflects and is correlated to the daily and seasonal activity of horseflies.

PUBLICATIONS DERIVED FROM THE THESES

Publications in English, peer-reviewed journals

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