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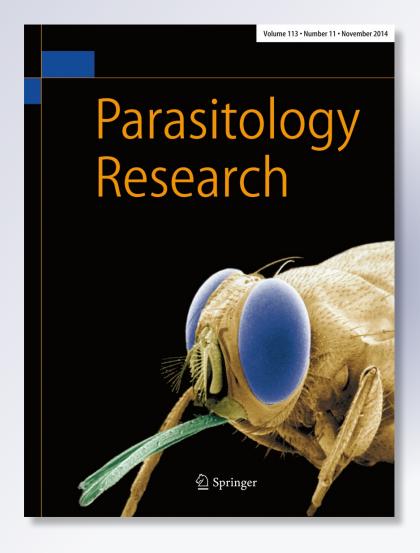
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Parasitology Research

Founded as Zeitschrift für Parasitenkunde

ISSN 0932-0113 Volume 113 Number 11

Parasitol Res (2014) 113:4251-4260 DOI 10.1007/s00436-014-4103-6





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ORIGINAL PAPER

Seasonality and daily activity of male and female tabanid flies monitored in a Hungarian hill-country pasture by new polarization traps and traditional canopy traps

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Received: 14 June 2014 / Accepted: 25 August 2014 / Published online: 6 September 2014 © Springer-Verlag Berlin Heidelberg 2014

Abstract Blood-sucking female tabanid flies cause serious problems for animals and humans. For the control of tabanids, the knowledge about their seasonality and daily activity is of great importance. Earlier, only traditional traps capturing exclusively female tabanids have been used to survey tabanid activity. The data of such temporal trapping do not reflect correctly the activity of male and female tabanid flies. Our

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major aim was to monitor the trapping numbers of male and female tabanids during a 3-month summer survey in Hungary. We used (i) conventional canopy traps with liquid traps on the ground beneath the canopy and (ii) L-shaped sticky traps with vertical and horizontal components. Our other goal was to compare the efficiencies of the two components of each trap type used. We observed two greater peaks of the trapping number of tabanids. These peaks started with increased catches of female tabanids captured by the canopy traps and the vertical sticky traps and ended with a dominance of male and female tabanids caught by the liquid traps and the horizontal sticky traps. The swarming periods were interrupted by rainy/cool days, when the number of tabanids decreased drastically. Among the 17 species, six dominated and composed 89.4 % of the captured tabanids: Haematopota pluvialis, Tabanus tergestinus, Tabanus bromius, Tabanus maculicornis, Tabanus bovinus and Atylotus loewianus. The number of water-seeking male and female tabanids rose up to 12–13 h and then decreased but had a secondary peak at about 17 h. The stochastic weather change and the communities of different species resulted in large standard deviations of the averaged number of tabanids in the course of a day. The horizontally polarizing (liquid and horizontal sticky) traps captured both male and female specimens and were about three times more efficient than the canopy and vertical sticky traps that caught only females. The results of the horizontal sticky traps corresponded to those of the liquid traps, while the catches of the vertical sticky traps corresponded to those of the canopy traps. The catches of the used trap types reflected well the species and water/ host-seeking composition of tabanids.

Keywords Horse fly · Tabanid fly · Tabanid trap · Canopy trap · Liquid trap · Sticky trap · Polarization · Polarotaxis · Water seeking · Host finding



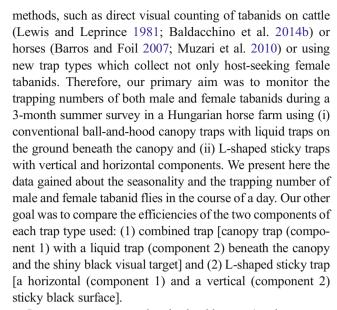
Introduction

Blood-sucking females of tabanid flies (order Diptera, suborder Brachycera, family Tabanidae) not only cause serious discomfort for humans and animals (Wilson 1968; Foil 1983; Baldacchino et al. 2014a) but also transmit the pathogens of numerous diseases (Foil 1989; Hall and Wall 2004; Lehane 2005). Tabanids disturb the grazing of large herbivores, like cattle, resulting in reduced meat and milk production in cattle farms (Hunter and Moorhouse 1976; Harris et al. 1987). An intense tabanid annoyance makes horse riding impossible outside or even may cause traumatic injuries by running the horses riot (Lin et al. 2011).

For the control of tabanids, the knowledge about their seasonal and daily activities is of great importance. Almost everything that we know about the seasonality and daily activity of these flies are based on their trapping (Hribar et al. 1991, 1992; Mihok 2002; Krcmar 2005a, b, 2013; Krcmar et al. 2005; Krcmar and Maric 2006; Mihok et al. 2006; Mohamed-Ahmeda and Mihok 2009; Mihok and Mulye 2010; Mihok and Lange 2012; Baldacchino et al. 2013a, b, c). On high-altitude Pyrenean summer pastures, for example, a maximal number of trapped tabanids were observed between the end of June and the beginning of August, and the main species had a peak of its trapping number at midday (Baldacchino et al. 2013c).

However, traps are different in their purpose, design and efficiency. For instance, traditional Manitoba type (ball-andhood) canopy traps (Muirhead-Thomson 1991) capture exclusively host-seeking female tabanids. On the other hand, the recently developed polarization tabanid traps—a liquid trap (Egri et al. 2013a), a horizontal sticky horsefly paper (Egri et al. 2013b) and a trap composed of a horizontal solar panel and a wire rotated by the electricity produced by the photovoltaics (Blahó et al. 2012a)—catch water-seeking male and female specimens. These traps utilize the polarization sensitivity of tabanid eyes (Wunderer and Smola 1986) and the positive polarotaxis of tabanids; that is, these flies are attracted to linearly polarized light: horizontally polarized light attracts water-seeking males and females (Horváth et al. 2008; Kriska et al. 2009), while linearly polarized light with high degrees of polarization attracts host-seeking females, independent of the direction of polarization (Egri et al. 2012a).

Until now, only traditional tabanid traps (mainly canopy traps and Nzi traps) capturing only host-seeking females have been used to survey the trapping numbers of these flies as a function of time. These conventional traps are usually believed to adequately reflect the actual tabanid abundance. The data of such temporal trapping are necessarily biased, because they do not reflect correctly the activity of male and water-seeking female flies, for instance. Due to this bias, tabanid surveys have to be completed with other sampling



In contrast to conventional tabanid traps (as the canopy trap, for example) catching only host-seeking females (Hribar et al. 1991; Foil and Hogsette 1994; Lehane 2005; Krcmar 2013; Baldacchino et al. 2014a), it is important to emphasize that our liquid traps and horizontal sticky traps capture water-seeking male and female tabanids. Thus, using these polarization-based traps makes it possible to monitor tabanids with quite other motivations (seeking water for drinking, bathing, mate finding and egg laying) than the motivation (seeking host for blood sucking) of tabanids caught by the conventional traps. In an insect survey, it is pertinent to use different traps capturing insects with various motivations and physiological status.

Materials and methods

Site of tabanid monitoring

Our 3-month monitoring was performed between 2 June and 28 August 2013 in a Hungarian horse farm at Szokolya (47° 52′ N, 19° 00′ E). The horse farm was located between two water reservoirs, a watercourse and a moorland, both were about 500–600 m far from the farm. The constant water supply and the 20 horses served an ideal biotop for tabanid flies where some earlier field experiments on tabanids were carried out (Kriska et al. 2009; Horváth et al. 2010a, b; Blahó et al. 2012a, b; Egri et al. 2012a, b, 2013a, b). The duration of cloudy, cool weather situations (when tabanid activity was practically zero) was registered continuously by a portable radio weather station (Conrad Electronic, Germany, equipment no. 672861) and a portable, automatic, imaging polarimetric cloud detector (Estrato Research and Development Ltd., Budapest).



Tabanid traps used

Polarization liquid trap

The used polarization liquid trap (Fig. 1a) is described in detail by Egri et al. (2013a). It was composed of a black circular plastic tray (diameter=50 cm) filled with 2 l tap water and 1 l vegetable oil. Since the surplus rainwater flowed out through an overflow tube, the trap kept its attractiveness to waterseeking polarotactic tabanid flies even after rainfall. Two liquid traps were placed 10 m apart from each other on the ground of a protected area of the horse farm, which were 4 and 12 m far from a line of trees/bushes. The liquids of the traps were changed once in a fortnight on average, especially after rainy days, or when the vegetable oil layer became too viscous (due to intense sunshine) on the water surface.

In the beginning of the monitoring (from 2 to 24 June 2013), the liquid traps were checked twice a day: at 1300 hours (local summer time=GMT+2 h, where GMT is Greenwich Mean Time) and at 2000 hours. From 25 June, when the greater swarming of tabanid flies started, the traps were checked hourly between 0800 and 2000 hours every day until the end (28 August 2013) of the monitoring. If at least one tabanid fly was found in the traps at 2000 hours, the traps were checked at nightfall (between 2100 and 2130 hours) again, and the captured tabanids were added to the 2000 hours' catch. Each day, the trapped tabanids were removed from the traps during the inspections (checkings) and were collected for later identification in two plastic bottles filled with 70 %

ethanol. Each day, one of the bottles contained the tabanids trapped until 1300 hours, while the other bottle had the tabanids collected after 1300 hours. Every day, a new pair of bottles was used. Thus, the hourly catch numbers were known every day, but the species could not be determined in situ. Taxonomical identifications happened only later in the laboratory, obtaining the numbers of species for each day before and after 1300 hours.

Conventional canopy trap

Two ball-and-hood canopy traps (each composed of a white canopy and a shiny black sphere as described by Egri et al. 2012a) were placed above the two polarization liquid traps (Fig. 1b), as it has been shown that the latter enhance the tabanid-capturing efficiency of the former (Egri et al. 2013a). The canopy traps were checked twice a day, at 1300 hours (celestial noon) and 2000 hours until the end of the survey. Each day, the tabanids captured by the canopy traps were removed from the traps during the inspections and gathered in two plastic bottles filled with 70 % ethanol. Other details of tabanid collection and handling were the same as in the case of the liquid traps.

Polarization sticky trap

Two horizontal and two vertical polarization sticky traps (socalled horsefly papers) were applied (Fig. 1c). These traps are described in detail by Egri et al. (2013b). The traps were

Fig. 1 Tabanid traps used in our whole-season monitoring. a Polarization liquid trap. b Two canopy traps. c An L-shaped polarization sticky trap composed of a horizontal and a vertical sticky panel. d Arrangement of the sticky, canopy and liquid traps in our experiment shown on a *map*

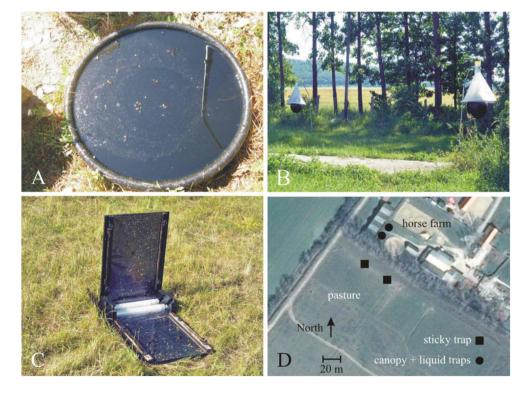
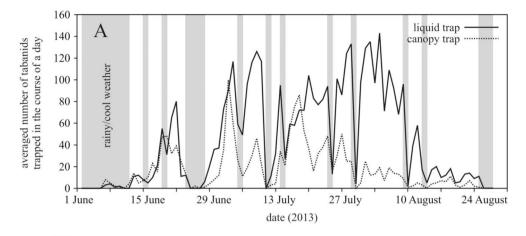
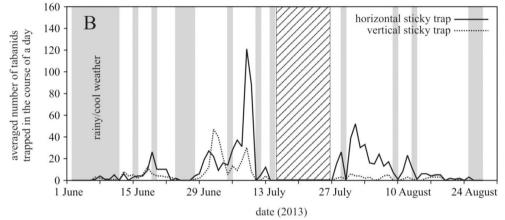




Fig. 2 Averaged number of tabanids trapped in the course of a day by the four trap types versus time during our whole-season monitoring. a Polarization liquid trap (continuous line) and canopy trap (dotted line). b Horizontal (continuous line) and vertical (dotted line) polarization sticky traps. The sticky traps were withdrawn from the field between 14 and 27 July in order to protect insectivorous birds during their nesting period, when there were no tabanid catches (column with tilted lines). Grey columns show the occurrence of rainy and/or cool days





placed 30 m apart from each other on the ground of a mown meadow and 4 m far from a line of trees/bushes (direction north) and the electric fence of a range (direction south), separately (Fig. 1d). A 1-week preliminary experiment showed that the vertical traps were most effective when their sticky side faced toward south (the range); thus the sticky vertical panel of both traps was facing to this direction during the 3-month monitoring.

The horsefly papers were checked twice a day, at 1300 and 2000 hours until the end of the survey. Tabanids were collected for taxonomical identification between 7 and 30 June 2013. Depending on the number of captured insects, the sticky horsefly papers were refreshed two to three times a week. These traps were withdrawn from the field between 14 and 27 July 2013 in order to protect insectivorous birds during their nesting period. After the activity peak of these birds, the sticky traps were set up again in the field to continue tabanid monitoring between 28 July and 28 August 2013. Other details of tabanid collection and handling were the same as in the case of the other trap types.

Identification of tabanids

The captured tabanids were identified (sex, species) according to the taxonomic keys of Majer (1987).



Statistical analysis

 χ^2 tests were performed to compare the numbers of tabanids captured by the different traps (Zar 2010) using the program Statistica 7.0.

Results

Tabanid seasonality monitored by different trap types

The first tabanid specimens were captured after a cold, rainy period in the beginning of June 2013, and at the end of August, no tabanids could be collected anymore. There were two greater peaks of the tabanid trapping number (Fig. 2a): (1) the first peak started between 12 and 24 June and (2) the second peak started on 29 June and ended on 17 August. The drop of the trapping number on 13 July was due to a rainy day. Both main swarming peaks started with increased catches of host-seeking females captured by the canopy traps and the vertical sticky traps and ended with a dominance of water-seeking males and females caught by the liquid traps and the horizontal sticky traps (Fig. 2). The swarming periods were interrupted by rainy/cool days, when the number of

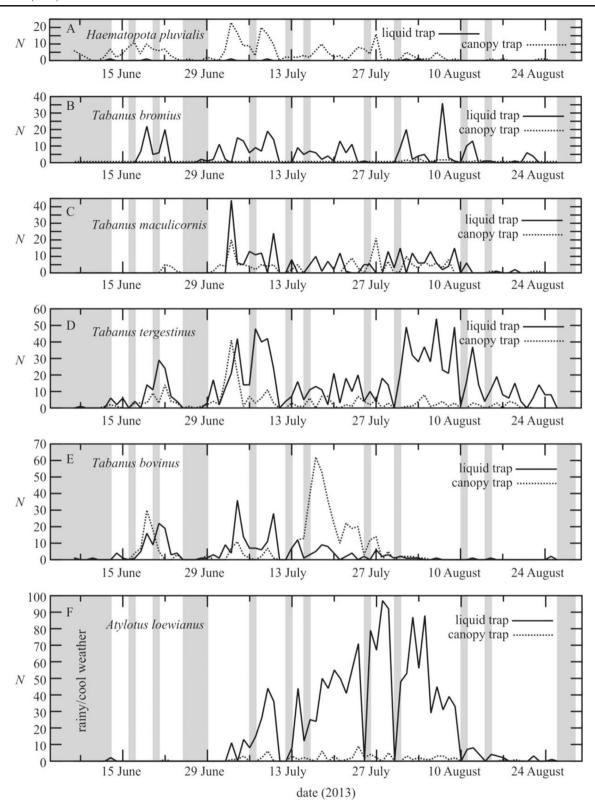


Fig. 3 Averaged number N of tabanids trapped in the course of a day versus time for the six most abundant tabanid species (Atylotus loewianus, Tabanus tergestinus, T. bovinus, T. maculicornis, T. bromius,

Haematopota pluvialis) captured by the canopy traps (dotted line) and the polarization liquid traps (continuous line)

tabanid specimens decreased drastically or neither water- nor host-seeking individuals were trapped at all. The first individuals of *Haematopota pluvialis*, *Tabanus tergestinus* and *Tabanus bromius* appeared at the start of June right on



the following day after a rainy week, while *Tabanus maculicornis*, *Tabanus bovinus* and *Atylotus loewianus* swarmed in bigger mass 2–3 weeks later. Although several different species coexisted during the season, when the number of individuals of a given species decreased, the individuals of another species began to increase: e.g. *T. bovinus* disappeared in August 2013 when *T. tergestinus* became abundant (Fig. 3).

Table 1 shows the numbers, sex and species percentages of tabanids captured by the canopy and liquid traps. The canopy traps captured practically only host-seeking females: there were only eight males (0.5 %) of the 1,507 flies. The liquid traps caught both males (26.3 %) and females (73.7 %). Remarkable differences were found between these two trap types, concerning not only the number of catches (3,871 tabanids captured by the liquid traps versus 1,507 by the canopy traps) but also the species. Among the 17 identified species, six dominated and composed 89.4 % of the captured tabanids. The seasonal patterns of the trapping number of the six dominant species can be seen in Fig. 3. A. loewianus was the most abundant species (1,585 specimens). It appeared in the beginning of July, and mostly, its water-seeking males and females were trapped for 6 weeks. T. tergestinus (1,350 specimens) occurred in the middle of June, and its swarming period lasted until the end of the season. First (in the second half of June), both water- and host-seeking T. tergestinus

individuals were trapped in huge numbers, but from July, water-seeking males and females dominated. A contrary tendency was observed for *T. bovinus* (760 specimens): host-seeking females dominated in the last 2 weeks of their swarming, which practically finished on the first days of August. The swarming of *T. maculicornis* (457 specimens) started with the appearance of some host-seeking females at the end of June, and after this short period, host- and water-seeking males and females were captured until the end of their swarming. *T. bromius* (350 specimens) was present all over the season, and interestingly, almost only water-seeking individuals were trapped. The situation was just the opposite in the case of *H. pluvialis* (308 specimens): the canopy traps were very effective in capturing host-seeking females, but the liquid traps caught only six water-seeking *H. pluvialis* tabanids.

Between 7 and 30 June 2013, we tested whether the quality of tabanids captured by the recently developed horsefly paper (shiny black sticky trap) was suitable for taxonomical identification. We found that 151 (90.4 %) of the collected 167 tabanids were intact enough and appropriate for sex and species identification purposes. Comparing these taxonomical data with those obtained from the canopy and liquid traps, we found that the results of the horizontal sticky traps corresponded to those of the liquid traps, while the catches of the vertical sticky traps corresponded to those of the canopy ones (Fig. 4). Although the catch numbers of the sticky traps

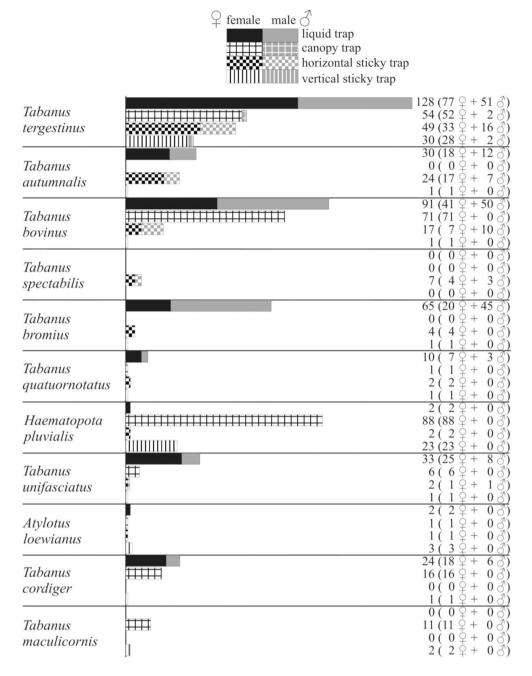
Table 1 Number of specimens, species, sex (female, male), species percentage (%) and male to female ratio (m/f) of tabanid flies captured by the canopy traps and the liquid traps during the whole season

Species	Canopy traps					Liquid traps				
	Female	Male	Sum	%	m/f	Female	Male	Sum	%	m/f
Tabanus bovinus	461	6	467	30.99	0.013	195	98	293	7.57	0.503
Haematopota pluvialis	302	0	302	20.04	0	5	1	6	0.15	0.200
Tabanus tergestinus	259	0	259	17.19	0	817	274	1,091	28.18	0.335
Tabanus maculicornis	182	0	182	12.08	0	201	74	275	7.11	0.368
Atylotus loewianus	74	0	74	4.91	0	1,134	377	1,511	39.03	0.332
Tabanus sudeticus	65	1	66	4.38	0.015	61	11	72	1.86	0.180
Tabanus cordiger	57	0	57	3.78	0	28	6	34	0.88	0.214
Tabanus unifasciatus	27	1	28	1.86	0.037	31	8	39	1.01	0.258
Tabanus glaucopis	24	0	24	1.59	0	0	0	0	0	_
Tabanus autumnalis	11	0	11	0.73	0	30	21	51	1.32	0.700
Tabanus spectabilis	11	0	11	0.73	0	0	0	0	0	_
Haematopota italica	7	0	7	0.46	0	0	0	0	0	_
Tabanus bromius	7	0	7	0.46	0	243	100	343	8.86	0.412
Tabanus quatuornotatus	7	0	7	0.46	0	104	49	153	3.96	0.471
Atylotus fulvus	3	0	3	0.20	0	0	0	0	0	_
Tabanus spodopterus	2	0	2	0.13	0	2	0	2	0.05	0
Tabanus bifarius	0	0	0	0	-	1	0	1	0.02	0
Total	1,499	8	1,507	100	_	2,852	1,019	3,871	100	-

Dominant species with percentages larger than 10 % are shown in italics, because our results and conclusions are mainly based on these dominant species



Fig. 4 Comparison of the total number and sex (female: ♀, male: ♂) composition of the 11 tabanid species captured between 7 and 30 June 2013 by liquid traps, canopy traps and horizontal and vertical sticky traps



were smaller than those of the liquid and canopy traps, the catches of the former reflected well the species and water/

host-seeking composition of the investigated tabanid population.

Table 2 Number and percentage (%) of tabanid flies captured by the different trap types in the forenoons and afternoons during the whole season. Time=local summer time=GMT+2 h, where GMT is Greenwich Mean Time

Trap type	Number of trapped tabanids						
	Forenoon (0800–1300 hours) (%)	Afternoon (1300–2000 hours) (%)	Sum (%)				
Liquid	2,023 (30.5)	1,851 (28.0)	3,871 (58.6)				
Canopy	628 (9.5)	886 (13.4)	1,507 (22.8)				
Horizontal sticky	549 (8.3)	351 (5.3)	900 (13.6)				
Vertical sticky	181 (2.7)	151 (2.3)	332 (5.0)				
Total	3,381 (51)	3,239 (49)	6,610 (100)				



Table 3 Statistical comparisons (χ^2 test) between the numbers of tabanids captured by the four different trap types in the forenoon and afternoon (Table 2). Using the observed forenoon (O_f) and afternoon (O_a) catch numbers (Table 2), the expected forenoon (E_f) and afternoon (E_a)

catch numbers were calculated as $E_f = (O_f + O_a) \cdot [t_f/(t_f + t_a)]$ and $E_a = (O_f + O_a) \cdot [t_a/(t_f + t_a)]$, because the forenoon sampling period was $t_f = 5$ h (0800–1300 hours), while the afternoon sampling period was $t_a = 7$ h (1300–2000 hours=7 h)

Trap type	Expected forenoon catches versus expected afternoon catches	χ^2 test	Significance
Liquid trap	$(2,023+1,851)\cdot(5/12)=1,614.2 \text{ versus } (2,023+1,851)\cdot(7/12)=2,259.8$	$\chi^2 = 177.5, df = 1, p < 0.0001$	Significant
Canopy trap	$(628+886)\cdot(5/12)=630.8$ versus $(628+886)\cdot(7/12)=883.2$	χ^2 =0.02, df=1, p=0.88	Not significant
Horizontal sticky trap	$(549+351)\cdot(5/12)=375$ versus $(549+351)\cdot(7/12)=525$	χ^2 =138.4, df =1, p <0.0001	Significant
Vertical sticky trap	$(181+151)\cdot(5/12)=138.3$ versus $(181+151)\cdot(7/12)=193.7$	χ^2 =22.6, df=1, p <0.0001	Significant

df degree of freedom

Trapping number of tabanids in the course of the day monitored by different trap types

The liquid traps and the two (horizontal and vertical) sticky traps captured statistically significantly more tabanids in the forenoon than in the afternoon, while there was no significant difference between the forenoon and afternoon catches in the case of the canopy traps (Tables 2 and 3). Figure 5 shows the average number of tabanids in the course of a day captured by the liquid traps calculated from the data of 38 days when tabanids were most abundant (more than ten trapped tabanids per day per liquid trap). The number of water-seeking tabanids raised until 12–13 h (celestial noon) and then decreased but had a secondary peak at about 17 h. The time of appearance of individuals of different tabanid species and sexes depended on the weather conditions.

According to Table 2, the liquid traps captured 2.6 times more tabanids (3,871, 58.6 %) than the canopy traps (1,507, 22.8 %). Similarly, the horizontal sticky traps caught 2.7 times more specimens (900, 13.6 %) than the vertical sticky traps (332, 5.0 %). Hence, the horizontally polarizing traps capturing water-seeking male and female tabanids were about three times more efficient than the traps catching only host-seeking females.

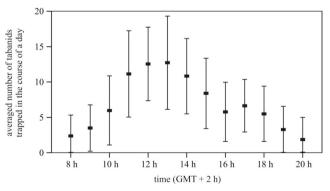


Fig. 5 Averaged number of tabanids trapped in the course of a day by the polarization liquid traps versus time. The averages (*black rectangles*) and standard deviations (*thin vertical Ts*) were calculated from the catches of 38 days when tabanids were the most abundant



Discussion

Seasonality of tabanids

Specimens of 17 horsefly species were caught during the whole season (Table 1) which number is only about one third of the 50 tabanid species found in Hungary (Majer 1987; Majer and Krcmar 1998). The six most frequent species (*A. loewianus*, *T. bovinus*, *T. tergestinus*, *T. maculicornis*, *T. bromius* and *H. pluvialis*) in our study have also been observed formerly (Egri et al. 2013a), and they occurred during the whole season (June–August) in an overlapping manner, but sometimes due to their different time of development, the populations of certain species completed/followed each other (Fig. 3).

The explanation of the relatively low 0.180≤m/f≤0.700 values of the male to female ratio in the case of different species captured by our liquid traps (Table 1) is not easy, especially if we consider that this ratio is about 1.0 at emergence (Baldacchino et al. 2014b), and the maintenance of annual tabanid populations requires successful oviposition by only 2% of females (Foil and Hogsette 1994). We suggest the following possible explanation: on the one hand, males need water only to drink and to control their body temperature, while beyond these two vital functions, females also need water to oviposit. This larger water demand of the females may be one of the main reasons why the liquid traps captured much more females than males.

Trapping number of tabanids in the course of the day monitored by polarization liquid traps

A correct estimation of the trapping number of tabanids in the course of a day should be based on the hourly catches of horizontally polarizing traps, because they capture both female and male water-seeking tabanids, rather than only host-seeking females (Egri et al. 2012a). The trapping number of water-seeking tabanids in the course of a day had a major peak around noon (12–13 h) and a minor peak at about 17 h (Fig. 5). This finding is very similar to the results obtained

in the sub-mountain region of the East Caucasus at altitudes of 900–1,000 m (Chvála 1979), though both the altitude and the climate in Caucasus are different from those of our study site at Szokolya, Hungary. Although the standard deviations in Fig. 5 are relatively large, the secondary peak of the trapping number around 17 h has also been observed in our earlier field experiments at the same study site (Horváth et al. 2010a, b; Blahó et al. 2012a, b; Egri et al. 2012a, b, 2013a, b).

Oliveira et al. (2007) and Van Hennekeler et al. (2011) found differences between the trapping numbers of different tabanid species in the course of a day in Queensland, Australia. It is well known that the flight activity of female tabanids is influenced by some meteorological factors (Burnett and Hays 1974; Dale and Axtell 1975; Alverson and Noblet 1977; Amano 1985). Depending on the changes of weather parameters, we also observed smaller or larger differences in the diurnal biological rhythm of the investigated tabanid species (Fig. 3). These results will be published in a separate paper. The stochastic change of the weather and the communities of different species resulted in the fairly large standard deviations of the average number of specimens in the course of a day in Fig. 5. On the basis of these results, we conclude that considering the trapping number of tabanids in the course of a day, riding out and/or keeping the horses on a meadow between 10 and 18 h (GMT+2 h) are not advisable during the peak swarming period of these flies.

Advantages and disadvantages of different tabanid traps

Our findings support that the success of defence against tabanids can be enhanced by a rational combination of the different trap types. Note, however, the total elimination of all tabanids from their biotop is impossible by traps alone. Beside their natural biological enemies, e.g. the microhymenopter *Telenomus* and *Trichogamma* larvae, dragonflies and insectivorous birds (Stone 1953), desiccation of the habitat of tabanid larvae can most effectively reduce the abundance of adult tabanids (Mikuska et al. 2012). Using repellents can serve only as a temporary protection against horseflies (Hall et al. 1998).

Our results presented here confirm that big differences can exist between the catches of tabanid traps and the tabanids collected on horses or cattle (Barros 2001). Due to these differences, the abundance of a tabanid population can be underestimated, even tabanid species can be hidden if only one trap type is used: male tabanids are not captured by ball-and-hood canopy traps, while certain species (like *H. pluvialis*, for instance) can be captured by liquid traps only occasionally and in a very small number.

Acknowledgments This work was supported by the grant TabaNOid 232366 (Trap for the Novel Control of Horse-flies on Open-air Fields) funded by the European Commission under the seventh framework

programme received by G. Horváth and G. Kriska. The financial support from the grant OTKA K-105054 (Full-Sky Imaging Polarimetry to Detect Clouds and to Study the Meteorological Conditions Favourable for Polarimetric Viking Navigation) received by G. Horváth from the Hungarian Science Foundation is also acknowledged. Gábor Horváth thanks the German Alexander von Humboldt Foundation for an equipment donation and a 3-month research fellowship (3.3-UNG/1073032 STP from 1 June to 31 August 2013 in the University of Regensburg). Many thanks to Csaba Viski (Szokolya, Hungary), who allowed our experiments on his horse farm. We also thank the constructive comments of two anonymous reviewers of the earlier drafts of this paper.

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