## ΝΟΤΕ

## The Effectiveness of Malaise Traps, H-Traps, and Sticky Traps for Collecting Horseflies (Diptera: Tabanidae)<sup>1</sup>

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The Malaise trap was initially constructed in the 1930s for collecting many groups of insects, including horseflies (Malaise, 1937, Entomol. Tidsk. 58: 148-160). This trap has undergone many changes and modifications (Townes, 1962, Proc. Entomol. Soc. Wash. 64: 253-262; Townes, 1972, Entomol. News 83: 239-247) that have increased its efficiency in collecting horseflies. In the last 50 yr, many trap types have been designed for collecting horseflies worldwide, and many studies have been conducted with different types of conventional traps in Europe, especially from faunistic perspectives. Malaise traps were used in several faunistic studies in Central Europe. During the 1970s, the efficiency of several types of Malaise traps for collecting horseflies in the United States was compared by Roberts (1970, Mosq. News 30: 567–571; Roberts, 1972, Mosq. News 32: 542– 547). Often Malaise trap efficiency was enhanced by different attractants (i.e., ammonia, carbon dioxide, or octenol) to significantly improve trap efficiency. Malaise traps designed by Townes (1962, 1972) have been used to evaluate the efficiency of different trap types for collecting of horseflies in eastern Croatia, with many of studies indicating that trap design affected quantity of specimens collected (Hribar et al., 1991, J. Am. Mosq. Contr. Assoc. 7: 657-659; Mihok, 2002, Bull. Entomol. Res. 92: 385-403). The first use of large black objects in traps to attract horseflies was demonstrated by Thorsteinson et al. (1966, Can. J. Zool. 44: 275-279). Allan and Stoffolano (1986, J. Med. Entomol. 23: 83-91) later reported that shiny black spheres attract horseflies. Recently, Otártics et al. (2019, Nat. Croat. 28: 51–61) used H-traps (with a shiny black beach ball) in a study of the horsefly fauna on an equestrian farm in Hungary, whereas Kline et al. (2018, J. Vector Ecol. 43: 63-70) used a similar H-trap in comparative trapping studies in Florida and North Carolina in the United States.

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## Fig. 1. Malaise trap (Townes design).

The attraction of horseflies to black spherical objects was attributed to horseflies being polarotactic insects that are attracted to horizontally and linearly polarized light (Horváth et al., 2008, Naturwissenschaften 95: 1093–1100; Egri et al., 2012a, Naturwissenschaften 99: 407–416). Females and males use polarotaxis governed by the horizontal direction of polarization to find water, whereas the polarotaxis based on the degree of polarization facilitates host finding by females (Egri et al. 2012a). Horváth et al. has since conducted many studies with recently developed polarization traps (Egri et al., 2013, Bull. Entomol. Res. 103: 665–674). The purpose of the study reported herein was to compare the efficiency of different trapping methods for attracting horseflies, including two types of traps with polarizing ability.

The following types of traps were used: (1) the Townes (1972) design Malaise trap henceforth referred to as Malaise trap, (2) H-traps, (3) and sticky traps. Malaise traps (Natural History Book Service, London, U.K.) were constructed of polyester mesh (Fig. 1). The central wall and two end walls were black, whereas the roof of trap was white. The trap length was 165 cm, the front side height was 170 cm, the back side height was 110 cm, and the width of both ends was 115 cm. A 170-cm-long aluminum pole on the front side of each Malaise trap was used as a prop, and the back side of trap was fixed with a 110-cm-long wooden pole. A 500-ml plastic collecting bottle was placed at the top of the trap. The opening width of the plastic tubes through which horse flies entered into the collecting bottle had a diameter of 2 cm. The collecting bottle contained 20 ml of 96% ethanol for killing horseflies.

The H-trap was supported by two poles (aluminum and iron) that were placed inside the trap (Fig. 2). A 70-cm-long pyramidal-shaped white cloth (tent) was



suspended from this construction, and a black rubber ball (60 cm diameter; Equi Team MT, Križevci, Croatia) was placed under the tent. The surface area of the sphere was  $1.1304 \text{ m}^2$ . The tent was fixed on the ground with ropes and metal wedges. The black sphere was suspended at a height of 125 cm. The distance between the lower part of the black ball from the ground was 65 cm, whereas the opening of the tent of the H-trap was 120 cm from the ground. A collecting cap was placed at the top of the upper white tent of the trap. The collecting cap was 40 cm high and 22 cm wide, with the radius 2 cm wider than the outer opening of the trap. The total height of H-trap was 230 cm.

The sticky trap was constructed using a black bucket (Equi Team MT) fixed to a bucket holder (iron pipe) by a rope through a hole in the center of the bucket (Fig. 3). The length of the rope was 30 cm. The iron pipe was bent at a 90-degree angle at the top so that the bucket could be attached to it. The 12-L black bucket (25 cm deep) was hung upside down. The height of the iron pipe from the ground was 125 cm, and the height of the lower edge of the black bucket from the ground was 70 cm. The black bucket was coated on the outside with a thin layer of sticky trap horsefly trap glue (RD Haaksbergen, Geesteren, Netherlands). The surface area of the black bucket (truncated cone) was 0.3204 m<sup>2</sup>.

Horseflies were collected during the summer months of 2020 (June, July, August) in three localities along the west banks of Danube River in eastern Croatia, in the area of the Batina forestry office that covers a total of 11,321 ha of wooded areas. The Zatonj site was in a forest of predominantly white willow (*Salix alba* L.) and black poplar (*Populus nigra* L.), the Himahat site was in the mixed forest of white willow, black poplar, ash (*Fraxinus* sp.), and oak (*Quercus* sp.), and the Monjoroš site was in a forest of oak and Dyer's greenweed (*Genista tinctoria* L.).

Nine traps (three traps of each type) were placed in forest meadows at each site. The traps were arranged in three rows. The rows were 10 m apart, and the distance between adjacent traps within each row was 10 m, with 20 m being the greatest distance separating two traps in the arrangement. Trap placement was rotated every day, and all traps were without any chemical attractants. Thirty total samplings were performed during this study, with 10 per month. The trapping period was from 0700 to 1900 hours on collection days. Trapped horseflies were stored in plastic containers in a freezer at  $-18^{\circ}$ C until identification, which was by the taxonomic keys of Krčmar et al. (2011, Period. Biol. 113: 1–61). A  $\chi^2$  test was used for comparison of the numbers of horseflies captured by different traps (P = 0.05). Standard deviations were conducted using the software program Statistica 7.0.

A total of 871 female horseflies, belonging to 16 species, were collected. The highest number of species (13) and specimens (329) were collected in the white willow and black poplar forest on the Zatonj locality, followed by the mixed forest of white willow, black poplar, ash, and common oak in the Himahat locality, with 320 collected specimens belonging to 12 species, and common oak wood and dyers green weed on the locality of Monjoroš with 222 collected horse flies and 12 identified species in the sample. *Tabanus* was the most frequently represented genus with five species, followed by the genera *Chrysops* with four species, *Hybomitra* and *Haematopota* with three species each, and *Atylotus* with one species. Overall, the most abundant species was *Tabanus bromius* (L.), with 32.5 % of the horseflies collected. *Tabanus tergestinus* (Egger) followed with 29.0%,



Species	Malaise Traps	H-traps	Sticky Traps	Total	χ²
Tabanus bromius L.	27	46	210	283	214.71
Tabanus tergestinus Egger	12	27	214	253	300.49
Haematopota pluvialis (L.)	11	63	69	143	0.26
Chrysops relictus Meigen	40	27	21	88	6.43
Hybomitra ciureai (Séguy)	1	12	8	21	NA
Haematopota subcylindrica Pandellé	0	18	2	20	NA
Tabanus sudeticus Zeller	5	7	6	18	NA
<i>Chrysops parallelogrammus</i> Zeller	2	13	0	15	NA
Tabanus autumnalis L.	1	5	4	10	NA
Haematopota italica Meigen	1	1	4	6	NA
Chrysops caecutiens (L.)	0	2	1	3	NA
Hybomitra bimaculata (Macquart)	1	1	1	3	NA
Tabanus maculicornis Zetterstedt	2	0	1	3	NA
Hybomitra ukrainica (Olsufjev)	0	2	0	2	NA
Atylotus loewianus (Villeneuve)	0	1	1	2	NA
Chrysops viduatus (F.)	0	1	0	1	NA
∑16	103	226	542	871	
Mean ± SD**	3.4 ± 4.8	7.5 ± 6.5	18.1 ± 14.2		

 Table 1. Species and numbers of horseflies collected using three different trap types in eastern Croatia.

NA, not analyzed.

\*Significant differences (P = 0.05).

\*\*Mean number of horseflies ( $\pm$ SD) per trap during the study.

*Haematopota pluvialis* (L.) with 16.4%, and *Chrysops relictus* Meigen with 10.1%, whereas the remaining 12 species made up 12.0% of the total collected (Table 1). However, there were differences in species abundance among localities. *Tabanus bromius* was the most abundant species of horseflies with 43.8%, in the woods of white willow and black poplar, whereas in the other two forest localities, the most abundant species was *T. tergestinus* with 44.4% and 36.0%, respectively. The most species (15) were collected by H-traps, followed by sticky traps with 13 and Malaise traps with 11 species. In all three study sites, sticky traps were the most effective in

collecting horseflies, followed by H-traps and the Malaise traps. Percentage of horseflies trapped in the sticky traps was 62.2%, whereas 26.0% were from H-traps and 11.8% from the Malaise traps (Table 1). In the sticky traps, 2.4 times more horseflies were collected compared with the H-traps and 5.3 times more than in Malaise traps. The different trap designs significantly differed in number of horseflies collected ( $\chi^2 = 353.31$ ; df = 3; P = 0.05). The sticky traps collected a mean  $\pm$  SD of 18 horseflies (18.1  $\pm$  14.2), whereas the H-traps and Malaise traps collected 7.5  $\pm$  6.5 and 3.4  $\pm$  4.8 horseflies/d, respectively. The largest number of horseflies was collected during July (542 specimens), followed by August with 253 specimens and June with 76 specimens. Sticky traps collected significantly more T. bromius and T. tergestinus females than H-traps and Malaise traps, whereas Malaise traps collected significantly more females of C. relictus than the other two trap types. Haematopota pluvialis was the third most commonly collected species. The highest number of this species was collected in the sticky traps, but analysis showed there was no statistically significant difference in the number of collected specimens between the sticky traps and H-traps ( $\chi^2 = 0.26$ ; df = 1; P = 0.05). The remaining 12 species were not analyzed further because of the low number of specimens collected.

In this study, the sticky traps and H-traps were more efficient than the Malaise traps in collecting horseflies. Results of this study correspond with the observations of Blahó et al. (2013, Physiol. Behav. 119: 168–174) and Egri et al. (2012b, J. Exp. Biol. 215: 736–745), where they showed that horseflies prefer sunlit dark targets or hosts. In addition to the dark-colored targets or traps, the polarizing ability is very important. Shiny black surfaces that reflect light with the highest degree of polarization attract blood-seeking female horseflies much more than matte black surfaces (Horváth et al., 2019, R. Soc. Open Sci. 6: 191119). Although the surface area of the black ball in the H-traps was larger than the surface area of the sticky black bucket, the sticky trap was more efficient in collecting horseflies. An explanation for these differences in the efficiencies of the sticky and H-traps in the number of horseflies collected is because of the glue used, as both traps have high polarizing ability. It was observed that female horseflies landed on black balls in Htraps, but they did not always fly into the collection cap. Unlike the H-traps, every landing of horseflies on a sticky trap ended with a catch. The Malaise traps without any attractants mainly intercept horseflies in flight and is probably the reason why this type of trap collected fewer horseflies in comparison with the other two trap types. However, Malaise traps collected many more specimens from different insect groups (i.e., Culicidae, Syrphidae, Muscidae, Calliphoridae, Ichneumonidae, Vespidae, Lepidoptera) than sticky and H-traps. The small number of horseflies collected in June was most likely affected by weather conditions in May, which was very cold, with a deviation of the mean monthly temperature of 1.8°C from the multiyear average on the area of eastern Croatia (data from the Croatian Meteorological and Hydrological Service).

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