# A new tabanid trap applying the modified concept of the old flypaper: Linearly polarizing sticky black surfaces as an effective tool to catch polarotactic horseflies

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## Abstract

Trapping flies with sticky paper sheets is an ancient method. The classic flypaper has four typical characteristics: (i) its sticky paper is bright (drab or white), (ii) it is strip-shaped, (iii) it hangs vertically, and (iv) it is positioned high (several meters) above the ground level. Such flypapers, however, do not trap horseflies (tabanids). There is a large need to kill horseflies with efficient traps, because they are vectors of dangerous diseases, and due to their continuous annoyance livestock cannot graze, horses cannot be ridden, and the meat and milk production of cattle is drastically reduced. Based on earlier findings on the positive polarotaxis (attraction to linearly polarized light) in tabanid flies and modifying the concept of the old flypaper, we constructed a new horsefly trap called as ”horseflypaper”. In four field experiments we showed that the ideal horseflypaper (1) is shiny black, (2) has an appropriately large (75 cm × 75 cm) surface area, (3) has sticky black vertical and horizontal surfaces in an L-shaped arrangement, and (4) its horizontal surface part should be on the ground to be the most efficient. Using imaging polarimetry, we measured the reflection-polarization characteristics of this new polarization tabanid trap. The ideal optical and geometrical characteristics of this trap revealed in field experiments are also explained. The horizontal part of the trap captures water-seeking male and female tabanids, while the vertical part catches host-seeking female tabanids.


**Key words**: horsefly, tabanid fly, insect trap, tabanid trap, sticky black surface, flypaper, light polarization, polarotaxis, polarimetry, water detection, host choice

## Introduction

It is a well-known fact that certain flies can be trapped by a sticky drab/white paper strip hanging vertically from the ceiling. This ancient trap is called the "flypaper" and is used from the beginning of the history of mankind (Beavis, 1988). Several different types of such flytraps are used to catch various insect species/groups for scientific purposes (Jactel *et al.*, 2006; Kamarudin and Arshad, 2006; Chadee and Ritchie, 2010; Faiman *et al.*, 2011), or for practical aims in the agriculture (Coli *et al.*, 1985; Stejskal, 1995; Cross *et al.*, 2006; Moreau and Isman, 2012). Depending on their application, the material (paper or plastic), colour (colourless or differently coloured), shape (e.g. rectangular or circular), stickiness (more or less tacky), alignment (vertical, tilted or horizontal) and position (e.g. laid on the ground, or onto an elevated substrate, or hanging high in the air) of these flytraps are different. Classic flypapers possess four typical characteristics: (1) their sticky paper is usually light drab or white, (2) their shape is a strip, (3) they hang vertically in the air, and (4) they are positioned several meters above the ground so they will not disturb people and/or animals in the vicinity.

Although these classic flypapers catch numerous different insect species, they do not trap tabanid flies. However, there is a large need to kill tabanids with efficient traps, because they are vectors of dangerous diseases (Foil, 1989; Luger, 1990; Maat-Bleeker and Bronswijk, 1995; Hall *et al.*, 1998; Sasaki, 2001; Lehane, 2005). Also, their continuous annoyance of livestock prevents grazing: horses cannot be ridden and the meat and milk production of cattle is drastically reduced (Hunter and Moorhouse, 1976; Harris *et al.*, 1987; Lehane, 2005). Several different trap types have been developed to reduce the number of tabanids (Malaise, 1937; Gressitt and Gressitt, 1962; Wilson *et al.*, 1966; Catts, 1970; Roberts, 1977; von Kniepert, 1979; Hayakawa, 1980; Wall and Doane, 1980; Hribar *et al.*, 1991, 1992; Moore *et al.*, 1996; Mihok, 2002). There are three main kinds of conventional tabanid traps: (i) flight interception traps, (ii) chemically baited canopy traps, and (iii) optically baited canopy traps. The common feature of these traps is that they are designed to attract female tabanids visually by shiny black objects and/or surfaces. It is generally believed that such black structures may simulate the dark silhouette of a host animal, and if they are flapping in the wind, their motion might mimic that of the host and attract female tabanids that want to suck blood (Thorsteinson *et al*., 1965, 1966; Lehane, 2005). The most frequently used visual target in these traps is a shiny black ball (e.g. a simple beach ball painted black). An important aspect of the optical attractiveness of such a ball due to linearly (or plane) polarized reflected light was recently revealed by Egri *et al*. (2012a).

Tabanids have positive polarotaxis, i.e. they are attracted to linearly polarized light (Horváth *et al*., 2008; Egri *et al*., 2012a), and this polarotactic behaviour can be used to develop new tabanid traps. Recently, Blahó *et al*. (2012a) designed such a polarization tabanid trap, the visual target of which is a horizontal solar panel (photovoltaics) attracting polarotactic tabanids by means of the horizontally polarized light reflected from the photovoltaic surface. The tabanids trying to touch or land on the photovoltaic trap surface are killed by the mechanical force of a wire rotated at a high speed with an electric motor powered by electricity produced by a solar panel.

The aim of this work is to describe another new tabanid trap that applies the modified concept of the old flypaper. We show here that linearly polarizing vertical and horizontal sticky black surfaces are an effective tool to catch polarotactic male and female tabanid flies. In field experiments we determined the ideal optical and geometrical characteristics of this sticky tabanid trap. Using imaging polarimetry, we measured the reflection-polarization characteristics of this trap to demonstrate the optical reason for the polarization attractiveness to tabanid flies. Our novel tabanid trap is a practical application of the knowledge accumulated in the last few years on the polarotaxis in tabanids (Horváth *et al*., 2008, 2010a,b; Kriska *et al*., 2009; Blahó *et al*., 2012a,b; Egri *et al*., 2012a,b).

## Materials and Methods

**Experiment 1 (greyness experiment)** was performed between 21 June and 12 September 2012 on a Hungarian horse farm at Szokolya (47$^o$ 52' N, 19$^o$ 00' E), where tabanids were in abundance. To study the influence of the brightness of horizontal and vertical sticky colourless tabanid traps on the attractiveness to tabanids, four pairs of plastic sheets (50 cm × 50 cm × 0.5 cm) were used (Fig. 1A, Supplementary Fig. S1) (any black plastic is suitable). One member of each test surface pair was horizontal, and the other member was vertical. The 1st test surface pair was black, the 2nd, 3rd and 4th pairs were dark grey, light grey and white, respectively. The centre of each vertical test surface was fixed at a height of 100 cm from the ground between two vertical metal rods hit with a hammer and driven into the ground. Each horizontal test surface laid onto the ground was fixed by four L-shaped metal hooks stuck into ground. The test surface pairs were set up 5 m apart from each other along a straight line. The horizontal distance was 50 cm between the horizontal and vertical members of each test surface pair. All eight test surfaces were simultaneously either in the sun or in the shade, and covered by a transparent, colourless, odourless, weather-proof insect-monitoring adhesive (BabolnaBio, Hungary). We periodically removed and counted the tabanids trapped by these sticky test surfaces. The surfaces were then cleaned with petrol, the order of the test surface pairs rotated according to a pre-determined randomized plan, and the adhesive was reapplied. The identification to species of the tabanids collected from these sticky surfaces was impossible, because their bodies were damaged seriously. It was obvious, however, that they were tabanids (Diptera: Tabanidae). In previous field experiments (Blahó *et al.*, 2012b; Egri *et al.*, 2012b) the following tabanid species were found to occur at the same study site with the use of self-made liquid-filled traps applied also in our earlier field experiments (Horváth *et al.*, 2008, 2010b, 2011; Kriska *et al.*, 2009; Blahó *et al.*, 2012a,b; Egri *et al.*, 2012a,b):

*Tabanus tergestinus*, *T. bromius*, *T. bovinus*, *T. autumnalis*, *Atylotus fulvus*, *A. loewianus*, *A. rusticus*, *Haematopota italica*.

**Experiment 2 (height experiment)** was performed between 21 June and 12 September 2012 at a distance of 100 m from the site of experiment 1. To study the influence of the height of horizontal and vertical sticky test surfaces on the attractiveness to tabanids, four pairs of black plastic sheets (50 cm × 50 cm × 0.5 cm) were used (Fig. 1B, Supplementary Fig. S2) (any black plastic is suitable). One member of each test surface pair was horizontal, while the other member was vertical. The 1st pair was set on the ground, while the 2nd, 3rd and 4th pairs were set at a height of 50, 100 and 150 cm from the ground level, respectively (these values refer to the height of the plane of the horizontal test surfaces and of the geometrical center of the vertical test surfaces). Each elevated horizontal test surface was fixed to four vertical metal rods driven with a hammer into ground. The horizontal test surface laid onto the ground was fixed by four L-shaped metal hooks stuck into ground. We periodically counted the tabanids trapped by these sticky black test surfaces. Other details of this experiment were the same as those of experiment 1.

**Experiment 3 (size experiment)** was performed between 21 June and 12 September 2012 at a distance of 100 m from the site of experiment 2. To study the influence of the size of horizontal and vertical sticky black test surfaces on the attractiveness to tabanids, four pairs of black plastic sheets were used (Fig. 1C, Supplementary Fig. S3) (any black plastic is suitable). One member of each test surface pair was laid horizontally on the ground, and the other member was set vertically with its center at 100 cm above the ground level. The dimensions of the sticky plastic sheets (thickness = 0.5 cm) of the 1st, 2nd, 3rd and 4th test surface pairs were 25 cm × 25 cm, 50 cm × 50 cm, 75 cm × 75 cm and 100 cm × 100 cm, respectively. We frequently counted the trapped tabanids. Other details of this experiment were the same as those of experiment 1.

**Experiment 4 (prototype experiment)** was performed between 28 July and 12 September 2012 at a distance of 100 m from the site of experiment 3. In this experiment we tested the functioning of a prototype of our new polarization horseflypaper (Figs. 1D and 2, Supplementary Fig. S4), the concept of which is patented in Hungary (patent number: P-07-00104, year of submission: 2007, year of publication: 2009). The prototype uses a roll of sticky insect-monitoring plastic foil (Rentokil FE-45 Luminos, width = 37 cm with a central 30 cm wide sticky band on its one side). It has a wooden base plate (43 cm × 57 cm) painted shiny black. At one short side of this base plate two perpendicular holders are mounted that have symmetrical engravings so that they can hold the roll of the sticky foil. The foil should be rolled out with the sticky side upside along the base plate until it covers the whole plate. Then the sticky foil is fixed with four screws along its two non-sticky long margins by two black wooden battens to the base plate. We used two optional supporting sticks that can be mounted to the prototype in a way that the sticky foil stands vertically instead of horizontally so that we can measure the tabanid-capturing efficiency of the trap for both orientations. The whole trap made of wooden boards and battens was painted shiny black to maximize the degree of polarization of trap-reflected light. Three different trap arrangements were used: (i) One vertical sticky black plate standing on the ground. (ii) One horizontal sticky black plate laid on the ground. (iii) An L-shaped pair with a vertical and a horizontal sticky black plate on the ground. These three traps were on the ground 5 m apart from each other along a straight line (Figs. 1D and 2). We periodically counted the trapped tabanids. Other details of this experiment were the same as those of experiment 1.

**Number of replications and sum of days of experiments**: In all four experiments we used sticky visual targets with different reflection-polarization characteristics to capture tabanids, which were frequently counted and removed, then the order of the test surfaces was randomly changed from a pre-determined plan. Our counting periods were nearly periodical.

The slight non-uniformity of these periods was purposive: after cool, rainy and windy weather the counting period was longer with a few days to compensate the decrease of tabanid flight activity. Because the trapped tabanids and all other insects were removed by frequent cleaning of the sticky test surfaces with petrol and the adhesive was reapplied, the newly arrived tabanids were not influenced by the presence of other trapped insects. Thus, the altered situation after each tabanid counting represented a new replication of a given experiment. In our experiments the number $R$ of replications during a test period composed of number of days $D$ were the following: experiment 1: $R$ = 12, $D$ = 84; experiment 2: $R$ = 12, $D$ = 84; experiment 3: $R$ = 12, $D$ = 84; experiment 4: $R$ = 7, $D$ = 47. These numbers of replications were large enough to detect statistical differences in the numbers of trapped tabanids.

**The reflection-polarization characteristics** of the test surfaces used in our experiments were measured by a self-constructed imaging polarimeter in the red (650 ± 40 nm = wavelength of maximal sensitivity ± half bandwidth of the CCD detectors of the polarimeter), green (550 ± 40 nm) and blue (450 ± 40 nm) parts of the spectrum (Figs. 2 and 3). The method of imaging polarimetry and our polarimeter have been described in detail by Horváth and Varjú (1997, 2004). Here we present only the polarization patterns measured in the blue part of the spectrum. Practically the same patterns were obtained in the red and green spectral ranges, because the test surfaces were colourless (white, grey, or black).

**Statistical analyses** (Mann-Whitney U test) were performed with the use of the program Statistica 7.0.

## Results

According to Table 1, in experiment 1 the black test surfaces captured the most tabanids (horizontal: 51.2%, vertical: 54.1%), the dark grey test surfaces of the same size were slightly less attractive (horizontal: 46.8%, vertical: 34.7%), while the light grey (horizontal: 1.9%, vertical: 1%) and white (horizontal: 0.1%, vertical: 10.2%) test surfaces of the same size were practically unattractive to tabanids. The horizontal black, dark grey and light grey test surfaces trapped 16.7, 23.8 and 33 times more tabanids than the corresponding vertical surfaces, respectively. On the other hand, the white vertical test surface trapped 10-times more tabanids than the horizontal white surface. From experiment 1 we conclude that (i) a sticky horizontal or vertical surface captures the most tabanids if it is black or dark grey, furthermore (ii) a horizontal black sticky surface on the ground can trap more than 15 times as much tabanids as a vertical one of the same size. These two differences are statistically significant (Supplementary Table S1).

Table 2 shows that in experiment 2 a horizontal sticky black test surface trapped tabanids practically (98.9%) only if it was on the ground. The horizontal black test surfaces of the same size at a height of 50, 100 and 150 cm captured only 0.7%, 0.2% and 0.2% of the total catches, respectively. On the other hand, the vertical sticky black test surfaces on (0 cm) and near (50 cm) the ground trapped much less (14.1% and 15.4%) tabanids than the more elevated (100 and 150 cm) vertical surfaces of the same size (37.8% and 32.7%). The horizontal test surface on the ground captured about 23 times more tabanids than the most effective vertical surface at 100 cm from the ground. From experiment 2 we conclude that a horizontal sticky black surface captures the significantly most tabanids if it is on the ground (Supplementary Table S2), when it traps more than 20 times as much tabanids as a vertical sticky black surface of the same size at a height of about 1 m from ground.

From Table 3 it is clear that in experiment 3 the number of trapped tabanids increased with the size of the sticky black test surface, independently of the surface orientation (horizontal or vertical). A horizontal test surface with a given size captured significantly much (6.3, 21.7, 15.3, 17.6 times) more tabanids than the corresponding vertical test surface of the same size (Supplementary Table S3). The surface density $\delta$ of trapped tabanids (number of cathes per 1 $m^2$) was maximal for both the horizontal ($\delta$ = 3541 /$m^2$) and the vertical ($\delta$ = 231 /$m^2$) test surfaces with dimensions of 75 cm × 75 cm. The surface densities $\delta$ of catches for the two smaller (25×25 $cm^2$ and 50×50 $cm^2$) vertical test surfaces were equal, while $\delta$ for the smallest (25×25 $cm^2$) horizontal test surface ($\delta$ = 912/$m^2$) was smaller than that for the second larger (50×50 $cm^2$) horizontal test surface ($\delta$ = 3128/$m^2$). The differences between the $\delta$-values for the horizontal or vertical test surfaces were, however, statistically not significant (Supplementary Table S3). From experiment 3 we conclude that the larger a horizontal or vertical sticky black surface, the greater the number of captured tabanids (Supplementary Fig. S5), and the ideal dimensions of horizontal and vertical sticky traps are 75 cm × 75 cm possessing maximum surface density of catches.

In experiment 4 (Table 4) the vertical sticky black surface of the new polarization tabanid trap (horseflypaper) captured significantly much less tabanids (5.4% and 5%) than the horizontal sticky black surface (38.3% and 51.3%). The horizontal surface $H_L$ of the L-shaped combined trap caught more tabanids (51.3%) than the single horizontal surface $H_S$ (38.3%), but this difference is not significant (Supplementary Table S4). The small difference between the catches of the vertical surfaces $V_L$ (combined: 5%) and $V_S$ (single: 5.4%) is also not significant (Supplementary Table S4). The combined trap captured more tabanids ($H_L+V_L$ = 56.3%) than the single horizontal ($H_S$ = 38.3%) and vertical ($V_S$ = 5.4%) traps together ($H_S+V_S$ = 43.7%), but this difference is not significant (Supplementary Table S4). The horizontal trap surfaces captured 7.0 and 10.2 times more tabanids than the vertical ones, which differences are statistically significant (Supplementary Table S4). From experiment 4 we conclude that the prototype of our new polarization tabanid trap functions excellently under field conditions (Figs. 1D and 2, Supplementary Fig. S4), and it is worth combining both the vertical and the horizontal sticky black trap surfaces in an L-shaped arrangement to maximize the tabanid catches. Due to practical reasons, the vertical part of the new trap stood on the ground, since it would be difficult to fix it at a wind-proof elevated position above the ground.

According to Fig. 2, the degree of linear polarization $d$ of light reflected from the vertical and horizontal sticky black surfaces of our new tabanid trap depends on the direction of view, but it is always high (70% < $d$ < 90%) near the Brewster angle [$\theta_{Brewster}$ = arc tan ($n$) = 56.3$^{o}$ from the normal vector of the plastic surface with a refractive index of $n$ = 1.5]. The direction of polarization of surface-reflected light is horizontal, if the plane of reflection is vertical. Thus, the horizontal surface part of the trap reflects always horizontally polarized light (represented by bright green and blue colours in row 3 of Fig. 2). If the plane of reflection is horizontal or tilted, the reflected light is vertically or obliquely polarized (represented by bright red and yellow colours in row 3 of Fig. 2). The consequence of these reflection-polarization characteristics is that a predominant percentage (> 90%) of the horizontal trap surface is always detected as water (represented by blue colour in row 4 of Fig. 2) by water-seeking polarotactic tabanid flies. Light with degrees of polarization $d$ > 20% and angles of polarization $80^{o} < \alpha < 100^{o}$ means water for polarotactic tabanids (Kriska *et al.*, 2009). On the other hand, depending on the direction of view, the vertical trap surface reflects light with horizontal, oblique or vertical direction of polarization with high degrees of polarization near the Brewster angle (Fig. 2). Thus the vertical horseflypaper attracts only host-seeking female tabanids.

Figures 3A-C show the reflection-polarization characteristics of a sunlit horizontal shiny black surface (plastic sheet, from which our test surfaces used in experiments 1-3 were composed) measured from three different directions of view relative to the sun, when the polarimeter saw perpendicular to the solar meridian (Fig. 3A), toward the anti-solar meridian (Fig. 3B) and toward the solar meridian (Fig. 3C). According to these polarization patterns, the light reflected from sunlit horizontal shiny black surfaces is always horizontally polarized, independently of the viewing direction with respect to the sun. The degree of polarization *d* of surface-reflected light is higher or lower, depending on the elevation of view, but it is always high enough to attract tabanids. Figures 3D-E show the reflection-polarization characteristics of a shady horizontal shiny black surface measured under a totally overcast sky from two different directions of view, when the polarimeter saw perpendicular to the solar meridian (Fig. 3D) and toward the anti-solar meridian (Fig. 3E). Under overcast sky conditions the illumination of this surface had approximately a rotational symmetry, and thus the reflection-polarization patterns of the surface were independent of the viewing direction relative to the invisible sun, as can also be seen in Fig. 3. Row 4 in Fig. 3 displays the areas of the horizontal shiny black surface detected as water by polarotactic tabanid flies. In row 4 of Fig. 3 we can see that the horizontal shiny black surface refected linearly polarized light with high degrees of polarization (represented by dark grey and black shades in row 2 of Fig. 3), and with exacty or nearly horizontal direction of polarization (represented by bright green and blue colours in row 3 of Fig. 3). The consequence of these polarizing characteristics is that the whole surface is sensed as water by polarotactic tabanids. This is the phenomenon that explains why a horizontal shiny (sticky) black surface is so strongly attractive to tabanids.

## Discussion

Our aim was to determine the ideal parameters of a new polarization tabanid trap applying the modified concept of the old flypaper. Based on the positive polarotaxis of female and male tabanid flies, we designed a trap composed of horizontal and vertical sticky black surfaces reflecting linearly polarized light with high degrees of polarization at the Brewster angle and thus attracting polarotactic tabanids. Like the classic flypaper, the new tabanid trap captures the attracted tabanids by the adhesive covering its surface. Because the target insects of this new sticky trap are tabanid flies, we call it ”horseflypaper” as an analogy of the classic name

”flypaper”. In three field experiments we determined the ideal brightness, height, orientation and size of this horseflypaper: According to experiment 1, the ideal horseflypaper is black, contrary to the classic flypaper being usually light drab or white. On the basis of experiment 2, the ideal black horseflypaper is either horizontal laid on the ground, or vertical at about 1 m from the ground, contrary to the classic flypaper, which always hangs vertically at several meters above the ground level. In experiment 3 we obtained that the ideal size of the black (horizontal or vertical) horseflypaper is about 75 cm × 75 cm, since this size ensures a maximum surface density of catches, contrary to the classic flypapers being usually a narrow strip.

Hence, changing the colour of an old vertically hanging flypaper from drab/white to black, its narrow strip shape to a 75 cm × 75 cm square, its height from several meters to about 1 m above ground, and its surface orientation from vertical to horizontal laid on the ground, we obtain an effective tool, the so-called ”horseflypaper” to catch polarotactic tabanid flies. Based on the results of experiments 1-3 we designed a prototype of this horseflypaper composed of a horizontal and a vertical sticky black surface in an L-shaped arrangement (Figs. 1D and 2). According to our experiences gathered in experiment 4, this prototype functioned well and captured tabanids efficiently under field conditions.

The fact that black is the ideal colour of the horseflypaper can be explained by the positive polarotaxis in tabanid flies. Tabanids are attracted to linearly polarized light, and the higher the degree of polarization, the larger the attractiveness (Horváth *et al*., 2008, 2010b; Egri *et al*., 2012a). Due to the rule of Umow (1905), the degree of linear polarization of light reflected from a shiny surface is the higher, the darker the surface. Thus, shiny black surfaces reflect light with the highest degrees of polarization. Consequently, such surfaces are the most attractive to polarotactic tabanids.

In experiment 4, the horizontal surface of the L-shaped combined horseflypaper caught 10.2 times more tabanids than the vertical surface (Table 4). In experiments 1-3 similar results were obtained (Tables 1-3): the horizontal black test surfaces trapped about 15-23 times more tabanids than the vertical ones. The reason for the phenomenon that horizontal sticky black surfaces on the ground can trap much more tabanids than vertical ones can be the following: Earlier, it has been shown that tabanids possess two different polarotaxis governed by different motivations (Egri *et al*., 2012a): (1) Female tabanids that look for host animals to suck blood are attracted to dark targets reflecting linearly polarized light with high degrees of polarization, independently of the direction of polarization. (2) Water-seeking male and female tabanids are attracted to horizontally polarized light, since such light means for them water, because they detect water remotely by means of the horizontal polarization of water-reflected light. Thus, the vertical sticky black test surfaces in our experiments trapped only those host-seeking female tabanids that wanted to suck blood for the development of their eggs. This host-finding period of female tabanids falls mainly on the beginning of the tabanid season. On the other hand, the horizontal sticky black test surfaces in our experiments trapped all male and female tabanids that wanted (i) to drink water, and/or (ii) to cool the body in water, and/or (iii) to mate at water, and/or (iv) to lay eggs into/near water (females only). Motivations (i) and (ii) are characteristic for the whole tabanid season, while motivations (iii) and (iv) are typical for the beginning-middle and the middle-end of the tabanid season, respectively. Due to these more or less permanent motivations the horizontal test surfaces kept their high attractiveness to male and female tabanids throughout the entire tabanid season, thus they captured much more tabanids than the corresponding vertical test surfaces.

The reason for the fact that in experiment 4 the horizontal surface of the L-shaped horseflypaper trapped only 10 times as many tabanids as its vertical surface, while in experiments 1-3 the horizontal test surfaces caught 15-23 times more tabanids than the corresponding vertical test surfaces, is that in experiment 4 the vertical component of the L-shaped horseflypaper stood on the ground, while the ideal height of a vertical sticky black tabanid-trapping surface is about 1 m. This is understandable, since black vertical surfaces imitate dark host animals which attract female tabanids that want to suck blood. A black vertical surface is better visible (and thus more attractive) from a more remote distance to flying host-seeking female tabanids if its height is approximately 1 m from the ground, rather than standing on the ground.

We experienced that a horizontal shiny black surface is attractive to tabanids only if it is on the ground. This can be explained in such a way that such a horizontally polarizing surface is sensed as water by flying tabanids, and the water surface is usually at the ground level. Tabanids seem to know this, and thus a horizontally polarizing surface that is elevated from the ground is not interpreted as water by tabanids. This is rather surprizing, since certain other aquatic insects are attracted to horizontally polarizing surfaces, even if these reflectors are elevated a few meters from the ground level. We mention, for instance, certain non-biting midges (chironomids) being also polarotactic (Lerner *et al*., 2008), and their females are attracted to horizontally polarized light reflected from test surfaces laid on car roofs (Horváth *et al*., 2011).

We experienced that the ideal size of both the vertical and horizontal surface components of the L-shaped combined horseflypaper is about 75 cm × 75 cm. Smaller or larger test surfaces trapped less tabanids per unit area (surface density in Table 3). As mentioned above, vertical dark surfaces mimick host animals for host-seeking female tabanids. A given tabanid species may prefer a vertical dark surface with a particular size, that corresponds with the average size of the preferred, or most abundant host animals. This preferred/optimal size may be tabanid species specific. In the habitat of our field experiments 1-4 and in the case of the tabanid species investigated (*Tabanus tergestinus*, *T. bromius*, *T.*

*bovinus*, *T. autumnalis*, *Atylotus fulvus*, *A. loewianus*, *A. rusticus*, *Haematopota italica*) the vertical size 75 cm × 75 cm was the most attractive to tabanids. Perhaps this is the most typical average size of host animals (horses, cattle, sheeps, dogs, humans) in this biotope.

On the other hand, the horizontal surface of our horseflypaper imitates a water surface for polarotactic water-seeking tabanids by the horizontally polarized reflected light. Considering drinking or body cooling by bathing, male and female tabanids may not prefer any water body of a particular size: tabanids could drink or bath practically in every water body. However, female tabanids may prefer an optimal size of water bodies as their egg-laying sites: too small water bodies can dry out quickly, hindering the development of tabanid larvae, while in too large water bodies fishes as predators can be dangerous to tabanid larvae. According to our experiment 3 (Table 3), in average the optimal size of oviposition sites seems to be about 75 cm × 75 cm for the tabanids investigated by us. This optimal size can, however, be species specific.

We did not study the optimal shape (e.g. triangular, rectangular, oval, or elongated) of the horseflypaper, because, in our opinion, this may not be an important variable. We have seen above that the vertical and horizontal surfaces of the horseflypaper imitate host animals and water bodies, respectively, to tabanids. Both objetcs as luring targets have usually a shape, the vertical and horizontal dimensions of which are nearly equal. Thus, apart from the extreme case of an elongated shape (e.g. a strip), the exact form of the tabanid-attracting target may be irrelevant. A vertical strip cannot mimic a typical host animal of tabanids (e.g. snakes are not typical tabanid hosts). Similarly, a horizontal strip does not imitate a characteristic egg-laying site of tabanids (e.g. a narrow flowing water trickle is surely not ideal/optimal for the development of tabanid larvae). Thus, the strip shape of the classic flypaper is not appropriate for an ideal/optimal horseflypaper.

The ideal trap surface of 75 cm × 75 cm has also the advantage that it can easily be handled manually when the trap is transported, set up, refreshed and maintaned in the field. The handling and maintaining of much larger trap surfaces would be rather difficult, while much smaller trap surfaces would be not enough efficient (Table 3, Supplementary Fig. S5).

It has been well documented that tabanids are generally attracted to dark, especially black objects, rather than bright ones (Granger, 1970; Roberts, 1970; Thompson and Pechuman, 1970; Anderson, 1985). Jones (1922), for example, reported on the attraction of male tabanids (mainly *Tabanus bromius*) to small dark pools of water. Roth and Lindquist (1948) observed that female *Chrysops discalis* were attracted to oviposit on sticky dark boards and stakes set in the water along the shore of a lake. Blickle (1955) created artificial dark pools of water at which he caught tabanids. Von Kniepert (1979) placed a black plastic sheet (1.5 m × 3 m) on the ground and caught by hand-netting several unspecified tabanid flies which were attracted to the plastic in a response identical to that of tabanids toward small water pools. Taylor and Smith (1989) captured *Tabanus sackeni* both at black plastic sheets and dark water puddles. Using unbaited black sticky boards, Moore *et al*. (1996) trapped male tabanids. Hall *et al*. (1998) captured both male and female tabanids (*Tabanus tergestinus* and *T. bromius*) by unbaited and odour-baited sticky black plastic sheets (30 cm × 30 cm) placed horizontally on the ground in a sheep pasture. Hence, in the past several researchers used horizontal sticky dark surfaces to capture tabanids. These sticky black test surfaces are the precursors of our new polarization tabanid trap, the horseflypaper. However, the cited researchers did not know the exact reason for the attractiveness of their shiny dark test surfaces to tabanids. In all the above-mentioned earlier experiments water-seeking tabanids were attracted by the horizontal polarization of reflected light, which polarotactic behaviour and its explanation was discovered by Horváth *et al*. (2008).

The effects of brightness and colour on the visual attraction of some tabanid species have been thoroughly studied (Tashiro and Schwardt, 1953; Bracken *et al*., 1962; Granger, 1970; Roberts, 1970; Browne and Bennett, 1980; Allan and Stoffolano, 1986; Allan *et al*., 1987, 1991; Moore *et al*., 1996; Sasaki, 2001). Depending on species, white or blue or red/brown or black was found to be the most attractive colour for host-seeking tabanids. Because the reflection-polarization characteristics of the coloured test surfaces/traps used in these experiments have never been measured, the relative role of polarization and colour remained unknown in the attraction of tabanids. In our field experiments 1-4 we used colourless (white, grey, black) test surfaces in order to eliminate the possible influence of colours on the attraction of tabanids.

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## References

Allan, S. A. and Stoffolano, J. G. (1986) The effects of hue and intensity on visual attraction of adult *Tabanus nigrovittatus* (Diptera: Tabanidae). *Journal of Medical Entomology* 23: 83-91

Allan, S. A.; Day, J. F. and Edman, J. D. (1987) Visual ecology of biting flies. *Annual Reviews of Entomology* 32: 297-316

Allan S. A.; Stoffolano, J. G. and Bennett, R. R. (1991) Spectral sensitivity of the horse fly *Tabanus nigrovittatus* (Diptera: Tabanidae). *Canadian Journal of Zoology* 69: 369-374

Anderson, J. F. (1985) The control of horse flies and deer flies (Diptera: Tabanidae). *Myia* 3: 547-598

Beavis, I. C. (1988) *Insects and Other Invertebrates in Classical Antiquity.* Exeter University Press, Exeter, UK, p. 288

Blahó, M.; Egri, Á.; Barta, A.; Antoni, G.; Kriska, G.; Horváth, G. (2012a) How can horseflies be captured by solar panels? A new concept of tabanid traps using light polarization and electricity produced by photovoltaics. *Veterinary Parasitology* 189: 353-365

Blahó, M.; Egri, Á.; Báhidszki, L.; Kriska, G.; Hegedüs, R.; Åkesson, S.; Horváth, G. (2012b) Spottier targets are less attractive to tabanid flies: on the tabanid-repellency of spotty fur patterns. *Public Library of Science ONE* (*PLoS ONE*) 7(8): e41138. doi:10.1371/journal.pone.0041138 + supporting information

Blickle, R. L. (1955) Observations on the habits of Tabanidae. *The Ohio Journal of Science* 55: 308-310

Bracken, G. K.; Hanec, W. and Thorsteinson, A. J. (1962) The orientation behavior of horseflies and deerflies (Tabanidae: Diptera). II. The role of some visual factors in the attractiveness of decoy silhouettes. *Canadian Journal of Zoology* 40: 685-695

Browne, S. M. and Bennett G. F. (1980) Color and shape as mediators of host-seeking responses of simuliids and tabanids (Diptera) in the Tantramar marshes, New Brunswick, Canada. *Journal of Medical Entomology* 17: 58:-62

Catts, E. P. (1970) A canopy trap for collecting Tabanidae. *Mosquito News* 30: 472-474

Chadee, D. D.; Ritchie, S. A. (2010) Oviposition behaviour and parity rates of *Aedes aegypti* collected in sticky traps in Trinidad. *Acta Tropica* 116: 212-216

Coli, W. M.; Green, T. A.; Hosmer, T. A.; Prokopy, R. J. (1985) Use of visual traps for monitoring insect pests in the Massachusetts apple IPM program. *Agriculture, Ecosystems and Environment* 14: 251-265

Cross, J. V.; Hesketh, H.; Jay, C. N.; Hall, D. R.; Innocenzi, P. J.; Farman, D. I.; Burgess, C. M. (2006) Exploiting the aggregation pheromone of strawberry blossom weevil *Anthonomus rubi* Herbst (Coleoptera: Curculionidae): Part 1. Development of lure and trap. *Crop Protection* 25: 144-154

Egri, Á.; Blahó, M.; Sándor, A.; Kriska, G.; Gyurkovszky, M.; Farkas, R.; Horváth, G. (2012a) New kind of polarotaxis governed by degree of polarization: attraction of tabanid flies to differently polarizing host animals and water surfaces. *Naturwissenschaften* 99: 407-416 + electronic supplement

Egri, Á.; Blahó, M.; Kriska, G.; Farkas, R.; Gyurkovszky, M.; Åkesson, S.; Horváth, G. (2012b) Polarotactic tabanids find striped patterns with brightness and/or polarization modulation least attractive: An advantage of zebra stripes. *Journal of Experimental Biology* 215: 736-745 + electronic supplement

Faiman, R.; Kirstein, O.; Moncaz, A.; Guetta, H.; Warburg, A. (2011) Studies on the flight patterns of foraging sand flies. *Acta Tropica* 120: 110-114

Foil, L. D. (1989) Tabanids as vectors of disease agents. *Parasitology Today* 5: 88-96

Granger, C. A. (1970) Trap design and color as factors in trapping the salt marsh greenhead fly. *Journal of Economical Entomology* 63: 1670-1673

Gressitt, J. C. L.; Gressitt, M. K. (1962) An improved Malaise trap. *Pacific Insects* 4: 87-90

Hall, M. J. R.; Farkas, R.; Chainey, J. E. (1998) Use of odour-baited sticky boards to trap tabanid flies and investigate repellents. *Medical and Veterinary Entomology* 12: 241-245

Harris, J. A.; Hillerton, J. E.; Morant, S. V. (1987) Effect on milk production of controlling muscoid flies, and reducing fly-avoidance behaviour by the use of Fenvalerate ear tags during the dry period. *Journal of Dairy Research* 54: 165-171

Hayakawa, H. (1980) Biological studies on *Tabanus iyoensis* group of Japan, with special reference to their blood-sucking habits (Diptera, Tabanidae). *Bulletin of the Tohoku Natural and Agricultural Experimental Station* 62: 131-321

Horváth, G.; Varjú, D. (1997) Polarization pattern of freshwater habitats recorded by video polarimetry in red, green and blue spectral ranges and its relevance for water detection by aquatic insects. *Journal of Experimental Biology* 200: 1155-1163

Horváth, G.; Varjú, D. (2004) *Polarized Light in Animal Vision – Polarization Patterns in Nature*. Springer-Verlag, Heidelberg - Berlin - New York, p. 447

Horváth, G.; Majer, J.; Horváth, L.; Szivák, I.; Kriska, G. (2008) Ventral polarization vision in tabanids: horseflies and deerflies (Diptera: Tabanidae) are attracted to horizontally polarized light. *Naturwissenschaften* 95: 1093-1100

Horváth, G.; Blahó, M.; Egri, Á.; Kriska, G.; Seres, I.; Robertson, B. (2010a) Reducing the maladaptive attractiveness of solar panels to polarotactic insects. *Conservation Biology* 24: 1644-1653 + electronic supplement

Horváth, G.; Blahó, M.; Kriska, G.; Hegedüs, R.; Gerics, B.; Farkas, R.; Åkesson, S. (2010b) An unexpected advantage of whiteness in horses: the most horsefly-proof horse has a depolarizing white coat. *Proceedings of the Royal Society B* 277: 1643-1650

Horváth, G.; Móra, A.; Bernáth, B.; Kriska, G. (2011) Polarotaxis in non-biting midges: female chironomids are attracted to horizontally polarized light. *Physiology and Behavior* 104: 1010-1015 + cover picture

Hribar, L. J.; LePrince, D. J. and Foil, L. D. (1991) Design for a canopy trap for collecting horse flies (Diptera: Tabanidae). *Journal of the American Mosquito Control Association* 7: 657-659

Hribar, L. J.; LePrince, D. J.; Foil, L. D. (1992) Ammonia as an attractant for adult Hybomitra lasiophthalma (Diptera: Tabanidae). *Journal of Medical Entomology* 29: 346-348

Hunter, D. M.; Moorhouse, D. W. (1976) The effects of *Austrosimulium pestilens* on the milk production of dairy cattle. *Austrian Veterinary Journal* 52: 97-99

Jactel, H.; Menassieu, P.; Vétillard, F.; Barthélémy, B.; Piou, D.; Frérot, B.; Rousselet, J.; Goussard, F.; Branco, M.; Battisti, A. (2006) Population monitoring of the pine processionary moth (Lepidoptera: Thaumetopoeidae) with pheromone-baited traps. *Forest Ecology and Management* 235: 96-106

Jones, H. (1922) Some notes on the habits of male Tabanidae. *The Entomologist* 55: 40-42

Kamarudin, N.; Arshad, O. (2006) Potentials of using the pheromone trap for monitoring and controlling the bagworm, *Metisa plana* Wlk (Lepidoptera: Psychidae) on young oil palm in a smallholder plantation. *Journal of Asia-Pacific Entomology* 9: 281-285

Kniepert, F. W. von (1979) Eine leistungsfähige Methode zum Fang Männlicher Bremsen (Diptera, Tabanidae). *Zeitschrift für angewandte Entomologie* 88: 88-90

Kriska, G.; Bernáth, B.; Farkas, R.; Horváth, G. (2009) Degrees of polarization of reflected light eliciting polarotaxis in dragonflies (Odonata), mayflies (Ephemeroptera) and tabanid flies (Tabanidae). *Journal of Insect Physiology* 55: 1167-1173

Lehane, M. J. (2005) *The Biology of Blood-Sucking in Insects*. 2nd ed., Cambridge University Press, Cambridge, UK

Lerner, A.; Meltser, N.; Sapir, N.; Erlick, C.; Shashar, N.; Broza, M. (2008) Reflected polarization guides chironomid females to oviposition sites. *Journal of Experimental Biology* 211: 3536-3543

Luger, S. W. (1990) Lyme disease transmitted by a biting fly. *New England Journal of Medicine* 322: 1752-1759

Maat-Bleeker F. and Bronswijk, van J. E. M. H. (1995) Allergic reactions caused by bites from blood-sucking insects of the Tabanidae family, species *Haematopota pluvialis* (L.). [abstract]. *Allergy* 50 (Supplement 26): 388

Malaise, R. (1937) A new insect-trap. *Entomologisk Tidskrift Stockholm* 58: 148-160

Mihok, S. (2002) The development of a multipurpose trap (the Nzi) for tsetse and other biting flies. *Bulletin of Entomological Research* 92: 385-403

Moore, T. R.; Slosser, J. E.; Cocke, J. and Newton, W. H. (1996) Effect of trap design and color in evaluating activity of *Tabanus abactor* Philip in Texas rolling plains habitat. *Southwestern Entomologist* 21: 1-11

Moreau, T. L.; Isman, M. B. (2012) Combining reduced-risk products, trap crops and yellow sticky traps for greenhouse whitefly (*Trialeurodes vaporariorum*) management on sweet peppers (*Capsicum annum*). *Crop Protection* 34: 42-46

Roberts, R. H. (1970) Color of malaise trap and collection of Tabanidae. *Mosquito News* 29: 236-238

Roberts, R. H. (1977) Attractancy of two black decoys and $CO_2$ to tabanids (Diptera: Tabanidae). *Mosquito News* 37: 169-172

Roth, A. R. and Lindquist, A. W. (1948) Ecological notes on the deer fly at Summer Lake, Oregon. *Journal of Economical Entomology* 41: 473-476

Sasaki, H. (2001) Comparison of capturing tabanid flies (Diptera: Tabanidae) by five different color traps in the fields. *Applied Entomology and Zoology* 36: 515-519

Stejskal, V. (1995) The influence of food and shelter on the efficacy of a commercial sticky trap in *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Journal of Stored Products Research* 31: 229-233

Taylor, P. D.; Smith, S. M. (1989) Activities and physiological states of male and female *Tabanus sackeni*. *Medical and Veterinary Entomology* 3: 203-12

Tashiro, H. and Schwardt, H. H. (1953) Biological studies of horseflies in New York. *Journal of Economical Entomology* 46: 813-822

Thompson, P. H. and Pechuman, L. L. (1970) Sampling populations of *Tabanus quinquetvittaus* about horses in New Jersey, with notes on the identity and ecology. *Journal of Economical Entomology* 63: 151-155

Thorsteinson, A. J.; Bracken, G. K.; Hanec, W. (1965) The orientation behaviour of horseflies and deerflies (Tabanidae: Diptera). III. The use of traps in the study of orientation of tabanids in the field. *Entomologia Experimentalis et Applicata* 8: 189-192

Thorsteinson, A. J.; Bracken, G. K.; Tostawaryk, W. (1966) The orientation behaviour of horseflies and deerflies (Tabanidae: Diptera). VI. The influence of the number of reflecting surfaces on attractiveness to tabanids of glossy black polyhedra. *Canadian Journal of Zoology* 44: 275-279

Umow, N. (1905) Chromatische Depolarisation durch Lichtzerstreuung. *Physikalische Zeitschrift* 6: 674-676

Wall, W. J.; Doane, O. W. (1980) Large scale use of box traps to study and control saltmarsh greenhead flies (Diptera: Tabanidae) on Cape Cod, Massachusetts. *Environmental Entomology* 9: 371-375

Wilson, B. H.; Tugwell, N. P. and Burns, E. C. (1966) Attraction of tabanids to traps baited with dry-ice under field conditions in Louisiana. *Journal of Medical Entomology* 3: 148-149

# Figure legends

**Figure 1**: Arrangements of the different sticky tabanid traps used in our four field experiments studying the influence of greyness (A), height (B), size (C) and alignment (D) of horizontal and vertical trap surfaces on the tabanid-capturing efficacy.

**Figure 2**: Colour photograph, patterns of the degree of linear polarization *d* and the angle of polarization α (clockwise from the vertical), and areas detected as water (for which the reflected light has the following characteristics: $d > 20\%$, $80^o < \alpha < 100^o$) of the horizontal and vertical sticky black surfaces of the prototype of the new polarization horseflypaper used in experiment 4. The patterns were measured in the blue part of the spectrum by imaging polarimetry from different directions of view relative to the trap surfaces. The traps were illuminated by direct sunlight and skylight from the clear sky. The angle of elevation of the optical axis of the polarimeter was $-35^o$ from the horizontal.

**Figure 3**: Colour photograph, patterns of the degree of linear polarization *d* and the angle of polarization α (clockwise from the vertical), and areas detected as water (for which the reflected light has the following characteristics: $d > 20\%$, $80^o < \alpha < 100^o$) of a horizontal shiny black test surface measured in the blue part of the spectrum when it was sunny (A, B, C) or shady (D, E) for different directions of view relative to the solar meridian. Towards SM: the polarimeter saw towards the solar meridian. Towards ASM: the polarimeter saw towards the anti-solar meridian. Normal to SM: the polarimeter saw normal to the solar meridian. The traps were illuminated by skylight from the totally overcast sky. The angle of elevation of the optical axis of the polarimeter was nearly $-35^o$ from the horizontal.

**Supplementary Figure S1**: Arrangement of the vertical and horizontal sticky test surfaces with different greynesses (black, dark grey, light grey, white) used in experiment 1.

**Supplementary Figure S2**: Arrangement of the vertical and horizontal sticky black test surfaces with different heights from the ground (0, 50, 100, 150 cm) used in experiment 2.

**Supplementary Figure S3**: Arrangement of the vertical and horizontal sticky black test surfaces with different dimensions (25 cm × 25 cm, 50 cm × 50 cm, 75 cm × 75 cm, 100 cm × 100 cm) used in experiment 3. The two smallest horizontal test surfaces (25 cm × 25 cm, 50 cm × 50 cm) laid on the grassy ground are almost invisible in this picture due to the perspective.

**Supplementary Figure S4**: Photographs of the vertically (A), horizontally (B) and horizontally and vertically (B) aligned sticky black surfaces of the prototype of the new polarization horseflypaper used in experiment 4. (D) Photograph of a horizontal sticky black test surface with numerous tabanid flies trapped.

**Supplementary Figure S5**: Photographs of the smallest (25 cm × 25 cm) and largest (100 cm × 100 cm) horizontal sticky black test surfaces used in experiment 3. The trapped tabanids (18 on the 25×25 $cm^2$ and 987 on the 100×100 $cm^2$) can be well seen.

# Tables

**Table 1**: Number of tabanids captured by the horizontal and vertical sticky black, dark grey, light grey and white test surfaces of the same size in experiment 1. The percentages given in brackets are calculated separately for the horizontal and vertical test surfaces.

| date (2012) | horizontal sticky test surfaces | | | | vertical sticky test surfaces | | | |
|---|---|---|---|---|---|---|---|---|
| | **black** | **dark grey** | **light grey** | **white** | **black** | **dark grey** | **light grey** | **white** |
| 28 June | 85 | 44 | 1 | 0 | 12 | 5 | 0 | 2 |
| 1 July | 16 | 84 | 1 | 0 | 11 | 11 | 0 | 5 |
| 10 July | 280 | 139 | 26 | 0 | 24 | 4 | 1 | 2 |
| 17 July | 141 | 175 | 0 | 0 | 2 | 1 | 0 | 0 |
| 25 July | 141 | 122 | 1 | 0 | 2 | 3 | 0 | 0 |
| 28 July | 1 | 5 | 0 | 0 | 1 | 3 | 0 | 0 |
| 8 August | 37 | 63 | 0 | 0 | 0 | 2 | 0 | 0 |
| 15 August | 42 | 42 | 1 | 0 | 0 | 4 | 0 | 0 |
| 23 August | 96 | 73 | 0 | 0 | 0 | 0 | 0 | 0 |
| 29 August | 30 | 37 | 0 | 1 | 0 | 0 | 0 | 0 |
| 4 September | 16 | 20 | 3 | 0 | 0 | 0 | 0 | 0 |
| 12 September | 1 | 5 | 0 | 0 | 1 | 1 | 0 | 1 |
| **sum** | **886 (51.2%)** | **809 (46.8%)** | **33 (1.9%)** | **1 (0.1%)** | **53 (54.1%)** | **34 (34.7%)** | **1 (1.0%)** | **10 (10.2%)** |

**Table 2**: Number of tabanids captured by the horizontal and vertical sticky black test surfaces positioned on the ground (0 cm) and at a height of 50, 100 and 150 cm from the ground in experiment 2. The percentages given in brackets are calculated separately for the horizontal and vertical test surfaces.

| **date (2012)** | **horizontal sticky black test surfaces** | | | | **vertical sticky black test surfaces** | | | |
|---|---|---|---|---|---|---|---|---|
| | **0 cm** | **50 cm** | **100 cm** | **150 cm** | **0 cm** | **50 cm** | **100 cm** | **150 cm** |
| 28 June | 162 | 0 | 0 | 0 | 8 | 4 | 5 | 6 |
| 1 July | 39 | 0 | 0 | 0 | 2 | 1 | 18 | 13 |
| 10 July | 428 | 3 | 0 | 0 | 5 | 10 | 29 | 20 |
| 17 July | 234 | 2 | 0 | 0 | 0 | 1 | 1 | 3 |
| 25 July | 136 | 1 | 0 | 0 | 2 | 4 | 2 | 3 |
| 28 July | 13 | 0 | 1 | 0 | 1 | 1 | 0 | 5 |
| 8 August | 25 | 3 | 2 | 2 | 3 | 2 | 0 | 0 |
| 15 August | 93 | 0 | 0 | 0 | 1 | 0 | 2 | 1 |
| 23 August | 136 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| 29 August | 40 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| 4 September | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12 September | 29 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| **sum** | **1350 (98.9%)** | **9 (0.7%)** | **3 (0.2%)** | **2 (0.2%)** | **22 (14.1%)** | **24 (15.4%)** | **59 (37.8%)** | **51 (32.7%)** |

**Table 3**: Number *N* of tabanids captured by the horizontal and vertical sticky black test surfaces with dimensions 25 cm × 25 cm ($A = 0.0625$ m$^2$), 50 cm × 50 cm ($A = 0.25$ m$^2$), 75 cm × 75 cm ($A = 0.5625$ m$^2$) and 100 cm × 100 cm ($A = 1$ m$^2$) in experiment 3. In brackets the numbers trapped by 1 m$^2$ are given. The surface density is $\delta = N / A$, where *N* is the total number of captured tabanids, and *A* is the surface area of the test surface.

| **date (2012)** | **horizontal sticky black test surfaces** | | | | **vertical sticky black test surfaces** | | | |
|---|---|---|---|---|---|---|---|---|
| | **25×25** | **50×50** | **75×75** | **100×100** | **25×25** | **50×50** | **75×75** | **100×100** |
| 28 June | 0 (0) | 53 (212) | 186 (331) | 319 (319) | 1 (16) | 4 (16) | 26 (46) | 27 (27) |
| 1 July | 0 (0) | 8 (32) | 41 (73) | 68 (68) | 0 (0) | 5 (20) | 30 (53) | 30 (30) |
| 10 July | 18 (288) | 265 (1060) | 578 (1028) | 987 (987) | 4 (64) | 11 (44) | 42 (75) | 70 (70) |
| 17 July | 2 (32) | 113 (452) | 321 (571) | 515 (515) | 3 (48) | 11 (44) | 8 (14) | 20 (20) |
| 25 July | 3 (48) | 89 (356) | 239 (425) | 407 (407) | 0 (0) | 0 (0) | 5 (9) | 14 (14) |
| 28 July | 1 (16) | 7 (28) | 3 (5) | 9 (9) | 0 (0) | 0 (0) | 2 (4) | 2 (2) |
| 8 August | 3 (48) | 50 (200) | 113 (201) | 328 (328) | 0 (0) | 2 (8) | 8 (14) | 18 (18) |
| 15 August | 10 (160) | 86 (344) | 190 (338) | 297 (297) | 0 (0) | 0 (0) | 2 (4) | 3 (3) |
| 23 August | 15 (240) | 76 (304) | 204 (363) | 342 (342) | 0 (0) | 0 (0) | 4 (7) | 4 (4) |
| 29 August | 4 (64) | 26 (104) | 69 (123) | 100 (100) | 1 (16) | 1 (4) | 0 (0) | 4 (4) |
| 4 September | 1 (16) | 8 (32) | 36 (64) | 58 (58) | 0 (0) | 0 (0) | 1 (2) | 0 (0) |
| 12 September | 0 (0) | 1 (4) | 12 (21) | 17 (17) | 0 (0) | 2 (8) | 2 (4) | 4 (4) |
| **sum *N*** | **57** | **782** | **1992** | **3447** | **9** | **36** | **130** | **196** |
| **density δ (1/m$^2$)** | **912** | **3128** | **3541** | **3447** | **144** | **144** | **231** | **196** |

**Table 4**: Number of tabanids captured by (i) the vertical sticky black surface standing on the ground, (ii) the horizontal sticky black surface laid on the ground, and (iii) the L-shaped combined sticky black trap with a vertical and a horizontal surface used in experiment 4. The percentages given in brackets in row 'sum' are calculated with pooling the data of all four test surfaces. The percentages given in brackets in row 'total' are calculated separately for the pair of the single vertical and horizontal surfaces, and the L-shaped combined trap.

| **date (2012)** | **sticky black surfaces of the new tabanid trap** | | | |
|---|---|---|---|---|
| | **single vertical** | **single horizontal** | **L-shaped combined** | |
| | | | **vertical** | **horizontal** |
| 31 July | 3 | 26 | 5 | 22 |
| 8 August | 11 | 45 | 4 | 99 |
| 15 August | 5 | 51 | 0 | 45 |
| 23 August | 1 | 22 | 5 | 30 |
| 29 August | 4 | 27 | 5 | 29 |
| 4 September | 3 | 23 | 5 | 35 |
| 12 September | 1 | 3 | 2 | 4 |
| **sum** | **28 (5.4%)** | **197 (38.3%)** | **26 (5.0%)** | **264 (51.3%)** |
| **total** | **225 (43.7%)** | | **290 (56.3%)** | |

# Supplementary Tables

**Supplementary Table S1**: Statistical comparisons (Mann-Whitney U test) between the numbers of tabanids trapped by the test surfaces of different greynesses but of the same size used in experiment 1 (Table 1). $H_b$: horizontal black, $V_b$: vertical black, $H_{dg}$: horizontal dark grey, $V_{dg}$: vertical dark grey, $H_{lg}$: horizontal light grey, $V_{lg}$: vertical light grey, $H_w$: horizontal white, $V_w$: vertical white.

| test surfaces | Mann-Whitney U test |
|---|---|
| $H_b$ *versus* $V_b$ | U = 14, z = 3.37, p = 0.0007 significant |
| $H_{dg}$ *versus* $V_{dg}$ | U = 3, z = 3.98, p < 0.0001 significant |
| $H_{lg}$ *versus* $V_{lg}$ | U = 41, z = 2.24, p = 0.02 significant |
| $H_w$ *versus* $V_w$ | U = 3.5, z = –2.02, p = 0.04 significant |
| $H_b$ *versus* $H_{dg}$ | U = 67, z = –0.28, p = 0.77 not significant |
| $H_{dg}$ *versus* $H_{lg}$ | U = 3, z = –4.02, p < 0.0001 significant |
| $H_{lg}$ *versus* $H_w$ | U = 41, z = 2.24, p = 0.02 significant |
| $V_b$ *versus* $V_{dg}$ | U = 62, z = –0.59, p = 0.55 not significant |
| $V_{dg}$ *versus* $V_{lg}$ | U = 20.5, z = –3.33, p < 0.0001 significant |
| $V_{lg}$ *versus* $V_w$ | U = 0.5, z = –2.24, p = 0.02 significant |

**Supplementary Table S2**: Statistical comparisons (Mann-Whitney U test) between the numbers of tabanids trapped by the test surfaces of different greynesses used in experiment 2 (Table 2). $H_0$: horizontal on the ground (0 cm), $V_0$: vertical on the ground (0 cm), $H_{50}$: horizontal at a height of 50 cm, $V_{50}$: vertical at a height of 50 cm, $V_{100}$: vertical at a height of 100 cm, $V_{150}$: vertical at a height of 150 cm.

| test surfaces | Mann-Whitney U test |
|---|---|
| $H_0$ *versus* $H_{50}$ | U = 0, z = 4.24, p < 0.0001 significant |
| $H_0$ *versus* $V_{100}$ | U = 0, z = 4.31, p < 0.0001 significant |
| $V_0$ *versus* $V_{50}$ | U = 40, z = –0.04, p = 0.96 not significant |
| $V_{50}$ *versus* $V_{100}$ | U = 54.5, z = –0.40, p = 0.69 not significant |
| $V_{100}$ *versus* $V_{150}$ | U = 37, z = –0.31, p = 0.76 not significant |

**Supplementary Table S3**: Statistical comparisons (Mann-Whitney U test) between the numbers *N* of tabanids trapped by horizontal (H) and vertical (V) test surfaces of the same size (25×25, or 50×50, or 75×75, or 100×100 $cm^2$), furthermore, between the surface densities $\delta = N / A$ (where *N* is the total number of captured tabanids, and *A* is the surface area of the test surface) of tabanids trapped by the test surfaces of different sizes used in experiment 3 (Table 3). The number next to the letter 'N' or 'δ' represents the side length (in cm) of the test surface (e.g., $H_{N75}$ corresponds to the total number of tabanids captured by the 75×75 $cm^2$ horizontal test surface).

| test surfaces | Mann-Whitney U test | |
|---|---|---|
| $H_{N25}$ *versus* $V_{N25}$ | U = 18.5, z = 2.45, p = 0.01 | significant |
| $H_{N50}$ *versus* $V_{N50}$ | U = 12.5, z = 3.45, p = 0.0005 | significant |
| $H_{N75}$ *versus* $V_{N75}$ | U = 12, z = 3.47, p = 0.0005 | significant |
| $H_{N100}$ *versus* $V_{N100}$ | U = 13, z = 3.41, p = 0.0007 | significant |
| $H_{\delta 25}$ *versus* $H_{\delta 50}$ | U = 36, z = –2.09, p = 0.04 | significant |
| $H_{\delta 50}$ *versus* $H_{\delta 75}$ | U = 65, z = 0.40, p = 0.69 | not significant |
| $H_{\delta 75}$ *versus* $H_{\delta 100}$ | U = 67, z = 0.29, p = 0.77 | not significant |
| $V_{\delta 25}$ *versus* $V_{\delta 50}$ | U = 21, z = –0.45, p = 0.65 | not significant |
| $V_{\delta 50}$ *versus* $V_{\delta 75}$ | U = 54, z = –1.05, p = 0.29 | not significant |
| $V_{\delta 75}$ *versus* $V_{\delta 100}$ | U = 69.5, z = –0.15, p = 0.88 | not significant |

**Supplementary Table S4**: Statistical comparisons (Mann-Whitney U test) between the numbers of tabanids trapped by the differently oriented sticky black test surfaces used in experiment 4 (Table 4). $H_S$: horizontal single sticky black surface, $V_S$: vertical single sticky black surface, $H_L$: horizontal sticky black surface of the L-shaped combined trap, $V_L$: vertical sticky black surface of the L-shaped combined trap.

| **test surfaces** | **Mann-Whitney U test** | |
|---|---|---|
| $H_L$ *versus* $H_S$ | U = 19, z = –0.70, p = 0.48 | not significant |
| $V_L$ *versus* $V_S$ | U = 20, z = –0.52, p = 0.60 | not significant |
| $H_S$ *versus* $V_S$ | U = 4, z = 2.63, p = 0.008 | significant |
| $H_L$ *versus* $V_L$ | U = 3.5, z = –2.52, p = 0.01 | significant |
| $H_S$+$V_S$ *versus* $H_L$+$V_L$ | U = 18, z = –0.83, p=0.41 | not significant |

**Revised Figure 1**

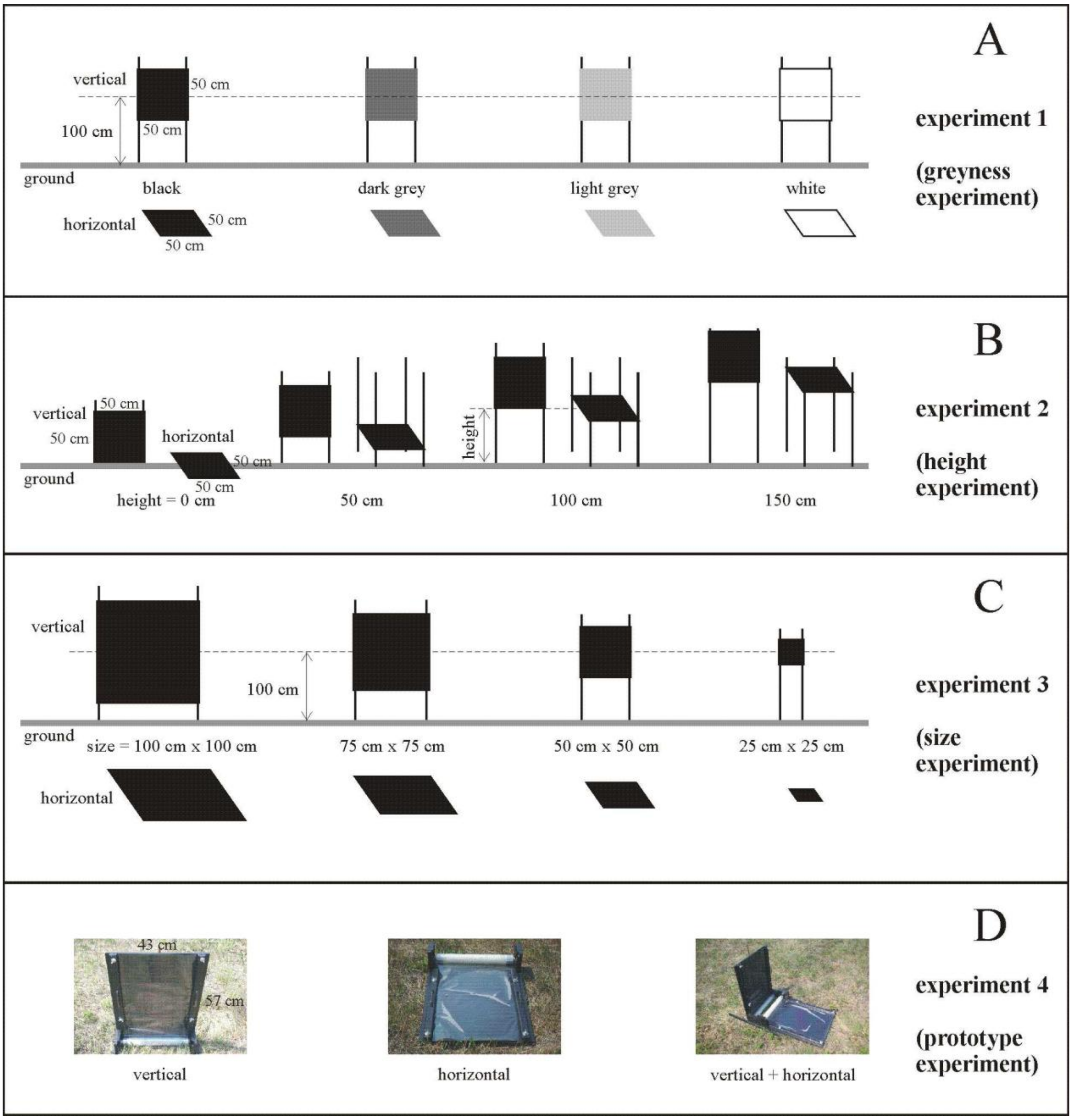



Figure 1

**Revised Figure 2**

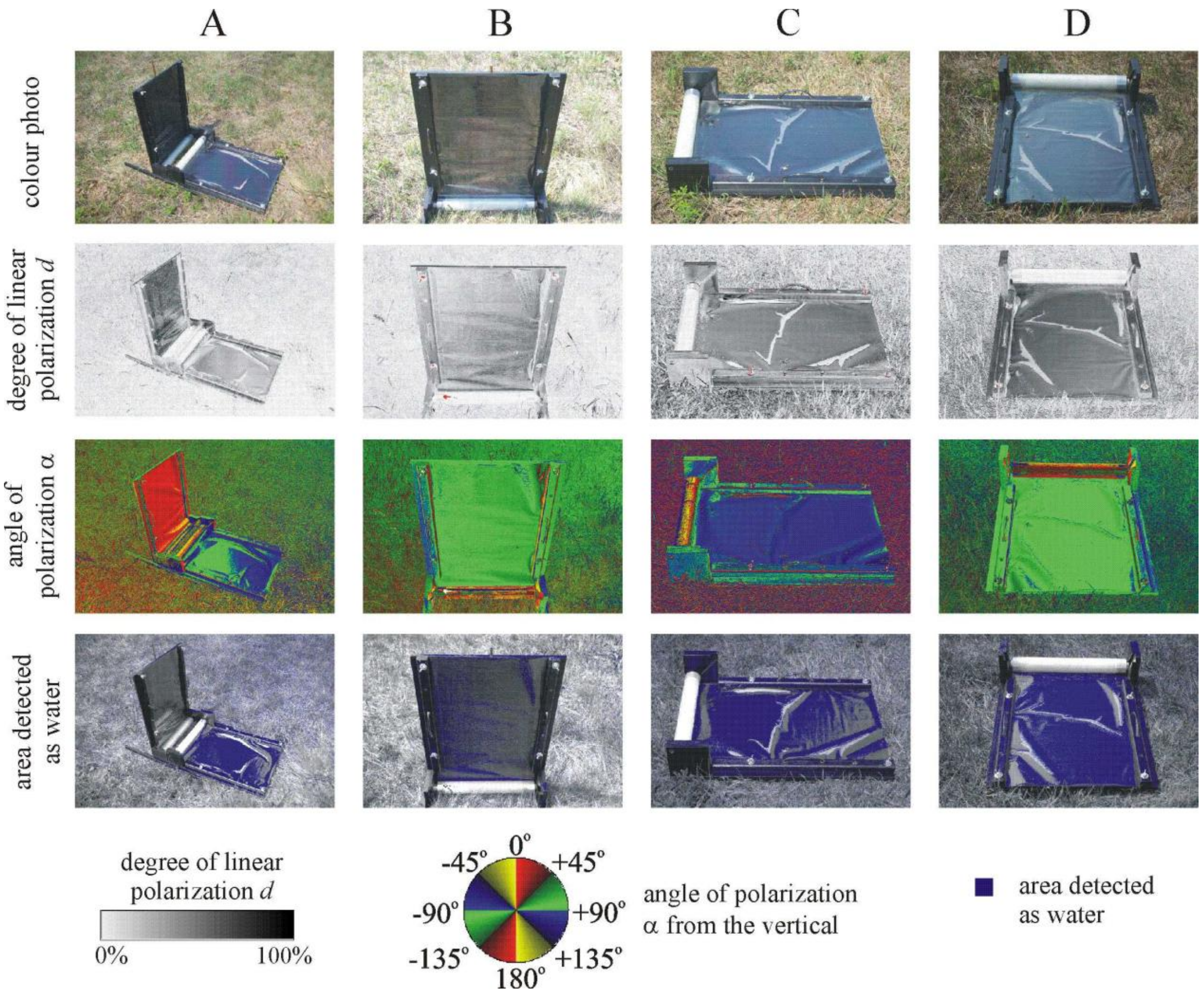



Figure 2

**Revised Figure 3**

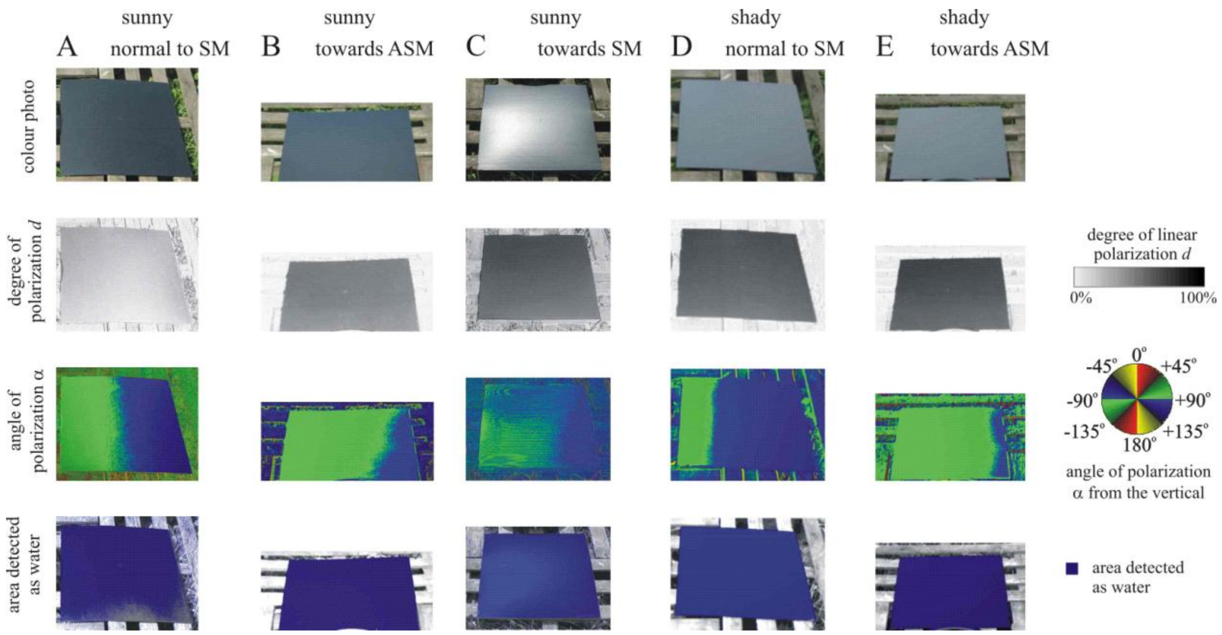
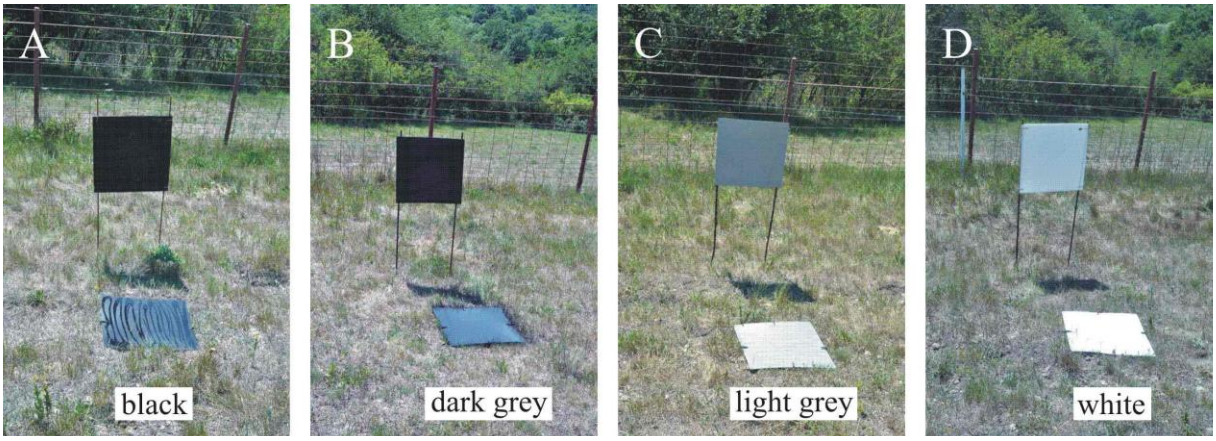
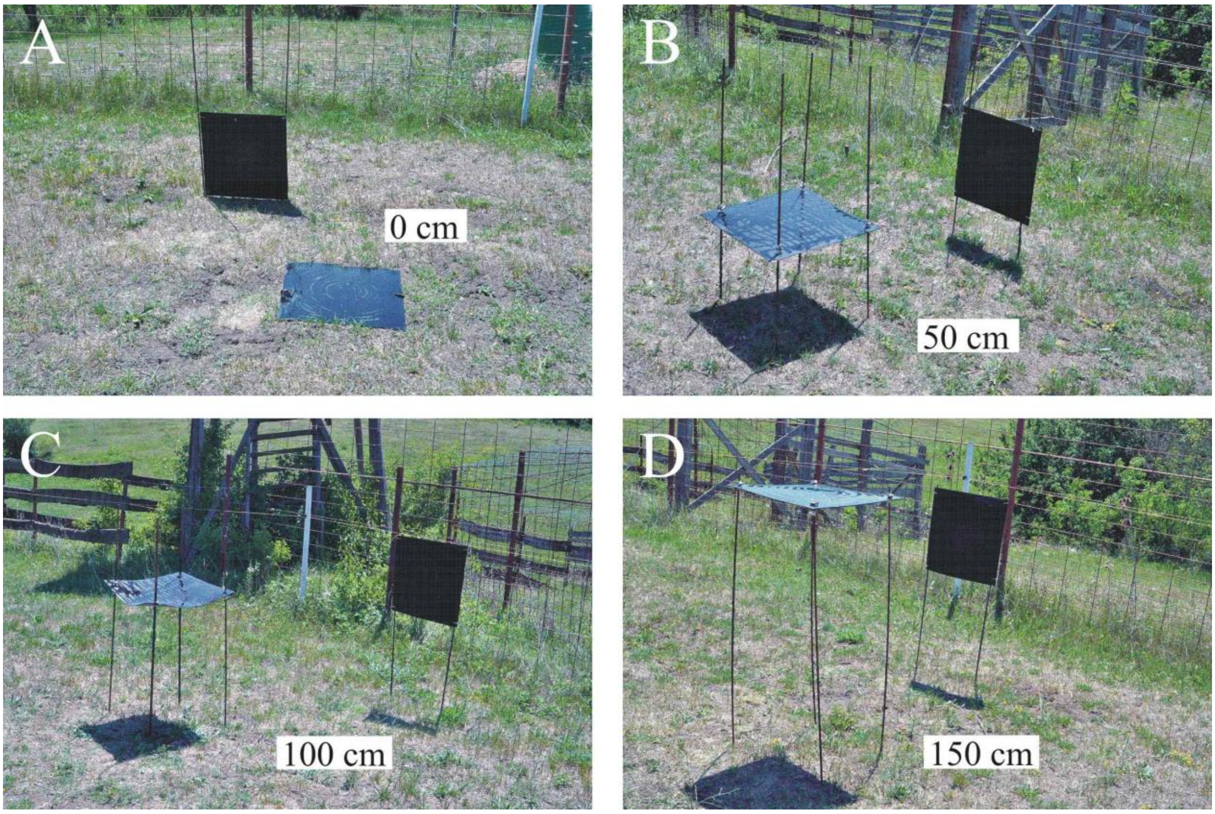



Figure 3

# Supplementary Figures



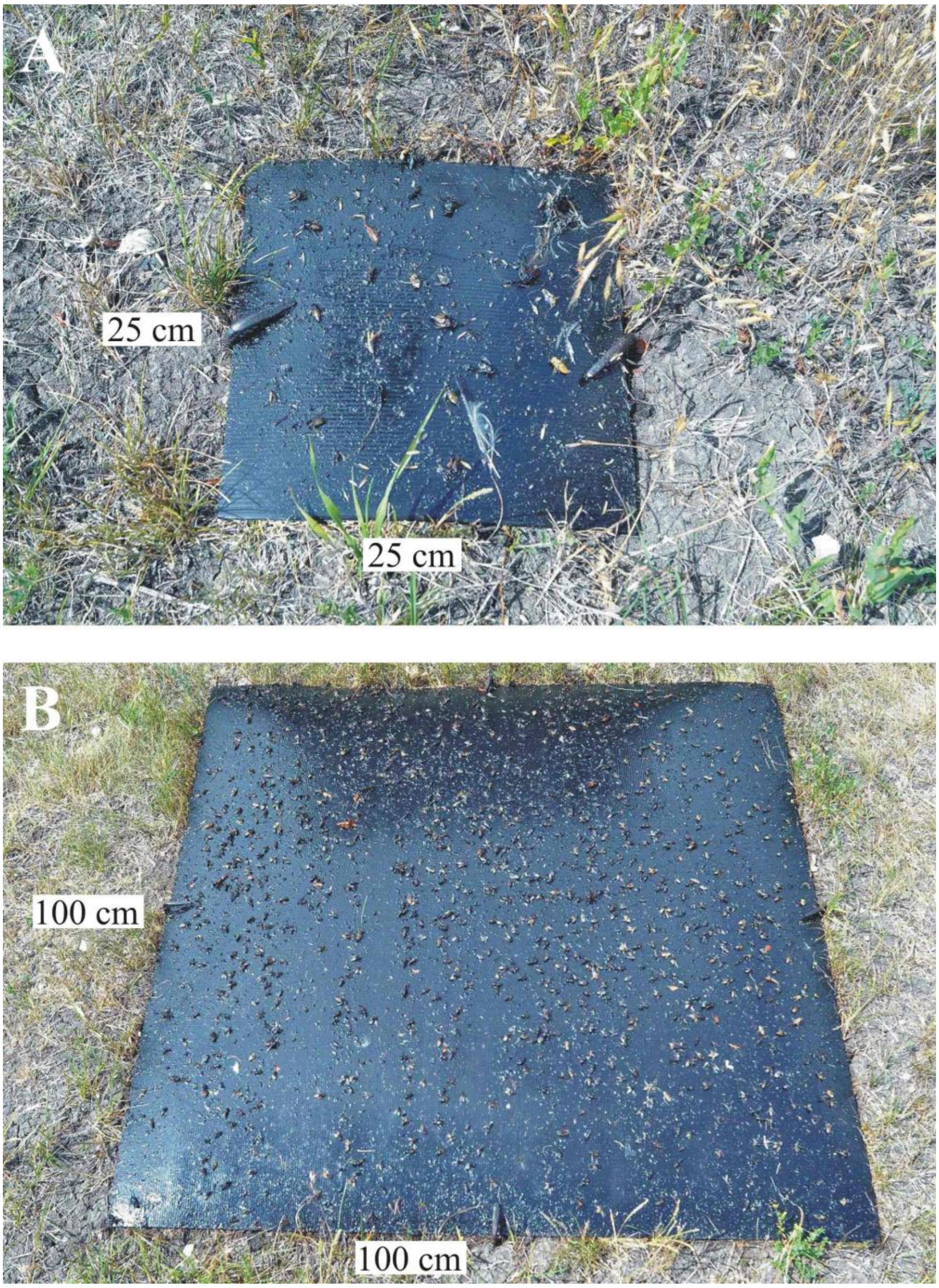

Supplementary Figure S1




Supplementary Figure S2


Supplementary Figure S3


Supplementary Figure S4




Supplementary Figure S5