Solar Radiation and Temperature Conditions as the Determinants of Occurrence of *Phlebotomus neglectus* Tonnoir (Diptera: Psychodidae)

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ABSTRACT

Sandfly species are rare Mediterranean fauna elements in Hungary. While *Phlebotomus* species prefer warm summers and mild winters, larvae and imagoes are sensitive to solar radiation, direct heat and low air humidity. It was proposed that diurnal patterns of solar radiation and temperature determine the breeding site occurrence of *Phlebotomus neglectus* Tonnoir. Light tapping, solar radiation and air temperature measurements were performed in Nagyharsány, South-Western Hungary. Hierarchical cluster and factor analyses were performed on solar radiation values according to the selected measuring which cover the floor of an abandoned quarry. A total of 202 *Phlebotomus neglectus* individuals were collected in 3 locations from the 6 trapping sites. Most of the individuals were trapped from one site. Characteristic differences were found in the diurnal patterns of temperature and solar radiation between the collecting sites. The non-shaded control areas received 15310 mmol m⁻² s⁻¹ solar irradiance in a sunny day, breeding sites only received 3794 mmol m⁻² s⁻¹solar irradiance per day. The major parts of the individuals were collected in the shaded environments with balanced microclimate. It was concluded that solar radiation is one of the most important factor of occurrence of breeding sites of *Phlebotomus neglectus*.

Key words: Phlebotomus, south western Hungary, trapping, summer, habitat.

INTRODUCTION

The causative agent of the protozoan parasitic infection of visceral leishmaniasis, *Leishmania infantum*, is transmitted by *Phlebotomus* species (Ferroglio *et al.*, 2005) in Mediterranean areas. Among the several sandfly taxa, *Phlebotomus neglectus* Tonnoir (1921) is a recognized vector of the canine leishmaniasis (Léger *et al.*, 1988). *Phlebotomus neglectus* is also a major vector of visceral leishmaniasis in humans (Chaniotis *et al.* 2000). Visceral leishmaniasis is the most severe form of leishmaniasis, which may be lethal if untreated. In Croatia, the neighboring country of Hungary, *Phlebotomus tobbi* Adler, Theodor and Lourie (1930) and *Ph. neglectus* are the most abundant sandflies (Bosnić *et al.*, 2006) while in Hungary the presence of *Ph. neglectus* and *Phlebotomus perfiliewi perfiliewi* Parrot (1930) and two other

sandfly species, *Phlebotomus papatasi* Scopoli (1786) and *Phlebotomus mascittii* Grassi (1908), were recorded Farkas *et al.* (2011), Tánczos *et al.* (2012). For the latter the vector status is not confirmed.

In general, the presence of *Ph. neglectus* is plausible in peridomestic and domestic sites Maroli *et al.* (2002) as bedrooms, old stone walls, but animal shelters (cow barns, corrals, chicken coops, pigpens; Velo *et al.*, 2005) or dog shelters are also characteristic sandfly habitats (Tarallo *et al.*, 2010; Chaniotis *et al.* 1994). Chaniotis *et al.* (1994) found *Ph. neglectus* populations in quarries. The small, drought-sensitive *Phlebotomus* eggs are deposited in small holes, cracks of natural or artificial walls or in the barks of trees (dendrotelmata). Larvae feed on organic materials and microorganisms alike fungi, decaying leaves, decomposing smaller animals and animal feces (El Sawaf *et al.*, 1991).

The abundance of sandfly species is tied to the presence of appropriate microclimatic conditions. All the four larval instars develop and feed on wet mats and require high humidity. Solar radiation has rapid and notable drying effect during summer daytime hours especially under dry meteorological conditions, which can result in unfavorable circumstances both for adult and immature ontogeny forms. As the flight radius of Phlebotomine sandflies may reach a maximum of one hundred meters per hour, the average flying distance can be much lower (Alexander, 1987; Doha *et al.*, 1991).

We resigned to testing the hypothesis of whether the solar radiation and temperature measurements at *Ph. neglectus*-occupied and -free sites differ significantly from each other.

MATERIALS AND METHODS

Study site

In this study, the effect of solar radiation and ambient air temperature on the breeding site occurrence of *Ph. neglectus* was studies in an abandoned quarry in South-West Hungary, near to the Croatian border (Fig. 1).

The Villány Hills are situated in the southernmost part of Hungary and made up Mesozoic carbonates. Szársomlyó, the highest peak of the Villány Hills (444 m), tower about 300m above the plain of Drava-river. The studied, abandoned limestone quarry was mined into the eastern end of the mountain. After stopping the mining activity in the 1970's, a sculpture park was established at the site. The orientation of the quarry is approximately west to east (Fig. 2A). The lowermost south foothill and the north part of the mountain are covered by deciduous forests (character species: *Tilia argentea* Moench (1785), *Carpinus betulus* L. (1753) and *Quercus petraea* ((Matt.) Liebl. (1784)) and the ridge and the south side of the mountain by rock grassland and karst forest vegetation. The most characteristic ligneous vegetation elements of the quarry are *Fraxinus ornus* L. (1753) and *Quercus pubescens* Willd. (1796) forming south-east Mediterranean karst type shrub forest vegetation (*Inulospiraefoliae-Quercetumpubescentis* association) mixed with rocky grassland on the south slope of the mountain (Nagy and Nagy 2000; Fig. 2B).



Fig. 1. Red spot marks the locality of the trapping site in Hungary.



Fig. 2A. The topographical model of Szársomlyó Mountain from south view. The site of the study can be found in the eastern endpoint of slope of the mountain (the right end of the mountain in the topographical reconstruction), Fig. 2B. The main vegetation zones in the eastern part of the mountain with the most successful trapping site 'i1'. Legend: fo=forest, ar=arable lands, kf=karst(shrub)forest, rg=rock grassland, qa=quarry.

No trappings were performed at the north and the east side of the quarry as there is no notable vegetation covered shelters for *Ph. neglectus*. At the north wall of the quarry mainly native cliff surfaces can be found with scattering low bush vegetation in some locations. The east part of the quarry is opened to the east foothill of the mountain. The central, secondarily leveled plain of the quarry is a reclaimed pit without ligneous vegetation and any shelters for sandflies. The south and west side of the quarry is covered by karst vegetation (mainly *Fraxinus ornus*). This part of the quarry is covered by large stone blocks. The presence of moss vegetation and *Ceterach javorkaeanum* (Vida) Soó (1963) fern indicates temporarily humid conditions in the base of the southern cliff wall.

Physical measurements and sandfly trappings

Twenty-seven measuring points and six trapping points were selected around the south and west wall in the quarry. Diurnal run of solar radiation and the relative temperature patterns were measured at hourly intervals (Fig. 3).



Fig. 3. The measuring points within the quarry with the characteristic environment of the central part of the quarry and the south, west and north walls. Black line show the north to south chain of the sites depicted in Fig.10.

Temperature was measured 2 m above soil level. Parallel with the solar radiation (US-SQS/L photometer and LI-1400 data logger product of the Heinz Walz GmbH). Since the used US-SQS/L is a submersible, rod-like sensor specially designed for measuring photosynthetically active radiation under water, subsequent calibration was necessary to gain valid PAR values. The measured brightness values were given in micromoles of photons in PAR spectrum per square meter per second (PAR mmol m⁻² s⁻¹) values. The measurment was performed on 28th July 2014, during atmospheric drought, in a completely cloud-free day when the number of daylight hours was 14.8 hours (about the mean of the daylight hours in summer at this latitude, which is 14.25 hours; Fig. 4). Since solar radiation in cloud-free and dry conditions depends on the angle of incidence of the sun (and eventually on the latitude: 45°51'N and 18°25'E), this single measurement can represent sufficiently the solar radiation conditions in summer under cloud-free conditions in the quarry.

Statistical analysis

Discriminant analysis was performed by the free PAST software, version 3.16 (Hammer, 2017). Daily means, maxima, minima and the standard variation of measured hourly radiation or temperature values were handled as the differentiating physical factors. Since the statistical software requires at least two nominal factor groups, the cardinals of the sites according to the corresponding quarry walls (categories: east, west or south) and the categories of trapping successfulness of the sites were

computed. The trapping frequency categories were the following: absence, p1 (> 1.15 mean trapping frequency) and p2 (\leq 1.15 mean trapping frequency).



Fig. 4. The hours of daylight in 28 July at 45.8° N according to the Daylight Hours Explorer software of the UNL Astronomy website.

CDC Miniature Light Traps (John W. Hock Company, FL, USA) were used, which are tested tools in entomological surveys of *Phlebotomus* species (Azizi *et al.*, 2006; Velo *et al.*, 2005), including *Ph. neglectus* (Maroli *et al.*, 2002). In six representative sites of the measurements (i1, g, k1, h1, f1, n1) sandfly trapping were performed in the summers of 2008, 2010 and 2014. Trapping were performed at the 'f1', 'g', 'h1', 'i1', 'k1' and 'n1' sites. In case of 'i1', 'k1' and 'l1' sites shaded, and vegetation-rich environment can be found in the close proximity of the trapping sites. The individuals were captured between 9 h to 11 h p.m.

RESULTS

A total of 202 *Ph. neglectus* individuals were collected in 3 locations from the 6 trapping sites (Fig. 5). The male/female gender ratio was 1.4. The highest number of individuals (64 individuals per night) were trapped at site 'i1', followed by sites 'g' and k1 (6-10 individuals per night). Not a single specimen was captured at sites 'h1', 'f1' and 'n1' (Table 1).



Fig. 5. A trapped male Ph. neglectus individual.

Site	Date	Male/Female	Total	Daily mean temperature (°C)
i1	09/07/2010 25/08/2010 24/08/2010 01/08/2008	51/10 0/1 0/2 63/61	61 1 2 124	21.38 20.35 23.19 25.29
mean of i1		37.6	188	
g	23/08/2010 24/08/2010 09/07/2010 25/08/2010 24/08/2010 23/08/2010	0/0 0/0 1/5 0/0 0/0 0/1	0 0 6 0 0 1	22.05 23.19 21.38 20.36 23.19 22.05
mean of g		1.16	7	
k1	14/08/2010	4/6	10	26.40
mean of k1		10	10	
h1	14/08/2014	0/0	0	20.36
f1	25/08/2010 24/08/2010 23/08/2010	0/0 0/0 0/0	0 0 0	20.36 23.19 22.05
mean of f1		0	0	
n1	27/07/2014 14/08/2014	0/0 0/0	0 0	20.79 21.35
mean of n1			0	
Total	-	112/80 (1.4)	202	

Table 1. The summary of trapping results.

The diurnal run of the solar radiation in the positive trapping sites

The sums of the diurnal solar radiation in the most successful trapping 'i1,' and the control, sunlit 'i2' sites were 5794 and 15013 PAR mmol m⁻² s⁻¹. Similar magnitude of difference was observed in case of the also successful trapping site 'k1' and the sunlit, control 'k2' points (1795 and 15185 PAR mmol m⁻² s⁻¹). While the control, sunlit 'i1', 'k1' points received 15099 PAR mmol m⁻² s⁻¹ average solar irradiance in the measuring day, the successful trapping site pairs received only 3794 PAR mmol m⁻² s⁻¹. The average irradiance and solar radiation reached their maxima mainly in the late morning hours at the successful trapping points. In contrast, the less successful 'g' trapping point received 15119 PAR mmol m⁻² s⁻¹ total irradiation during the same time. Between 7 and 11 h a.m. the most successful 'i1' and 'k1' trapping sites received only the 38.3% of the solar radiation of the other measuring points and 38.8% of the sunlit control pairs, but between 1 and 4 p.m. this value was only 9.6% of the non-successful trapping site pairs. The standard deviation of the mean diurnal solar radiation at the other points was 2.3 times higher (577 PAR mmol m⁻² s⁻¹ (Fig. 6).

The standard deviation of the solar radiation at the most successful 'i1' trapping site was 436.5 mmol m⁻² s⁻¹ between 6 a.m. and 8 p.m., while at the less successful trapping f1, g sites this value was 667.4 and 659.9 mmol m⁻² s⁻¹ during the same period.

Averaging the hourly solar radiation values, characteristic differences were found between the daytime solar irradiation run of the successful and non-successful trapping

sites. In case of the very successful i1 trapping site the mean maximum of solar irradiance was measured at 9:00 a.m. with 1263.5mmol m⁻²s⁻¹. In the non-successful sites, a radiation plateau was observed between 9:00 a.m. to 3:00 p.m with a 1359.8 mmol m⁻²s⁻¹ mean solar radiation value. At the less successful g and f1 sites the radiation plateau was observed between 10 a.m. and 3. p.m. with 1359.8 mmol m⁻²s⁻¹ value. At site i1 the total mean cumulative hourly irradiation value was 5793.6 mmol m⁻²s⁻¹ between 6:00 a.m. and 8:00 p.m., in case of the g and f1 sites and the non-successful sites this value was 14111.2 and 14839.2, respectively. Comparing the mean irradiation of the successful and the non-successful trapping sites it was found that at the most successful groups. Comparing the irradiation of the most successful i1 and the non-successful groups. Comparing the irradiation of i1 site was only the 39% of the mean of the other non-successful groups between 6:00 a.m. and 8:00 p.m. (Fig. 7).



Fig. 6. The interpolated solar irradiation patterns in the different measuring times in the quarry from the early morning hours to the evening (light gray tones: sunny sites, dark gray tones: shaded sites; data in PAR mmol m⁻² s⁻¹).

The diurnal run of air temperature

Temperature sum in the studied day varied between 447.9 and 422°C. The difference between the maxima and minima at 'i1' and 'k1' sites were 8.6 and 8.3°C.

At site 'a' this value was 10.3°C. The mean difference between the maximum and minimum temperature of the other sites was 9.1°C. Slight differences were observed in the diurnal run of the ambient temperature when comparing the successful and the non-successful trapping sites. In the successful trapping sites, the averages of the mean, the minimum, the maximum and the standard deviance values of air temperature were somewhat lower (27.5, 22.7, 31.0 and 2.51 °C) than the similar values measured at the other sites (27.9, 23.1, 32.1 and 3.11 °C). The same kind of values of the point 'g' were higher than the average values of the non-successful sites (28.2, 22.6, 32.9, 3.25 °C) except the minimum temperature. The ambient air temperature showed a characteristic change according to the mean direction of the solar radiation. In summer, the western wall of the guarry receives first the direct solar radiation in early morning which results in a the rapid, but short increase of the air temperature. In the rest of the morning time the surface of the western and southern walls - the habitats of Ph. neglectus - showed moderately warm temperature values, although in the late morning due to the appearing shades, the temperature began to decrease in the successful trapping points. In the morning and afternoon hours the geometrical axis of the guarry showed the greatest temperature values due to the infrared radiation and the focusing effect of the guarry walls. In the evening, the highest temperature values were measured near to the southern and south-western part of the quarry (Fig. 8).



Fig. 7. The daytime run of solar radiation in the different site groups in 28th July 2014.

The diurnal run of the air temperature in the successful trapping sites was characterized by a rapid elevating phase in the morning hours, and a slow increase in the early afternoon hours followed by a prolonged decreasing phase in the evening. The measured prolonged evening decrease of the ambient temperature can be the consequent on the heat-emitting effect of the quarry walls. Figure 11 shows the relative temperature patterns of air temperature in the quarry in the different hours of the day (Fig. 9).

Depicting the 'e1', 'f2', 'g', 'h2' and 'i1' sites in a map, these sites form a quasi-north to south cross-section chain within the quarry. Averaging the temperature differences from the hourly means of the five sites, opposite temperature profiles were fund in the comparison of the diurnal period 11. 0.m. to 3 p.m. and 7 p.m. to 11 p.m. In late

morning and the first half of afternoon, the shaded south part of the quarry was cooler than the central and north part. In contrast, from the evening to night hours, the south points are warmer than the north ones. At evening and night, the difference was more expressed: for example, the mean differences between the northernmost site 'e1' and the southernmost 'i1' in 11. 0.m. to 3 p.m. was only 0.8°C, but in 7 p.m. to 11 p.m. it was 1.7°C (Fig. 10).



Fig. 8. The diurnal run of air temperature in the different site groups in 28th July 2014.



Fig. 9. The changing pattern of the ambient temperature iatdifferent sites of the quarry from the morning hours to mid-afternoon hours (between 6 a.m. to 3 p.m.) and the evening hours (between 6 p.m. to 11 p.m.).





Results of discriminant analyses

Both the discriminant analysis of irradiation and temperature values showed that the most successful trapping 'i1' and k1' sites are well separated from the other measuring and trapping points of the quarry. The less successful trapping site 'g' was wedged into the main point population in both cases. According to the discriminant analyses result of radiation values, the sites show more notable segregation from the major point population as it can be seen in case of the temperature values (Figs. 11 and 12).

DISCUSSION

Geographic expansion of sandfly species is predicted as a consequence of the anthropogenic climate change in Europe (Trájer *et al.*, 2013). Though an extrazonal population of *Ph. neglectus* can be found in Budapest in Hungary due to the urban heat island effect, but the occurrence of the species at the studied site, Nagyharsány is the northernmost observed occurrence of the species in the Carpathian basin (Bede-Fazekas and Trájer, 2015; Trájer *et al.*, 2014). The studied quarry represents a special habitat of *Ph. neglectus* since quarries are known, but not too frequent habitats of sandflies (Chaniotis *et al.* 1994). In summer, the southern wall of the quarry of the Nagyharsány Sculpture Park is shaded, however due to the east-west axis; the main part of quarry is sunlit. From late morning the southern wall become shaded and the trend increase during the afternoon. Before sunset, the western wall of the quarry receives the less amount of solar radiation. Each of the successful trapping sites represents shaded or semi-shaded environments with relatively low diurnal solar irradiation. The successful trapping sites receive sunshine during summer in the early morning hours causing the short warming of the air in the southern wall of the quarry.

In the rest of the daytime the southern wall was the coolest part of the quarry, but in the evening hours the air at inner part of the west and south wall are the warmest sites within the quarry due to the heat-reflection effect.



Fig. 11. The discriminant analysis result of the irradiation values. A: absence (empty circles), P1: mean number of trapped sandflies more than 1.16 (black, filled rhombuses), P2: mean number of trapped sandflies less than 1.16 (dark gray rhombus).



Fig. 12. The discriminant analysis result of the temperature values. A: absence (empty circles), P1: mean number of trapped sandflies more than 1.16 (black, filled rhombuses), P2: mean number of trapped sandflies less than or equal to 1.16 (dark gray rhombus).

The heat reflection of the walls can also be observed in the midday hours in the axis of the quarry. Since *Phlebotomus* species are Mediterranean fauna elements,

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the warmer early morning and evening air temperature which does not accompanied with higher solar irradiation can be advantageous for the thermophile *Ph. neglectus*. The occurrence of *Ph. neglectus* was restricted to the sites with the most stable and temperatures which is in accordance with the fact that sandflies are highly sensitive to sudden temperature changes. Trájer *et al.* (2014) found that the shaded, moist surface of channel protrusions and cracks provide similar, preferable breeding sites for *Phlebotomus* species. Sandflies can rest in humid, cold places during the daytime in warm summer days retreating into cracks and crevices during the daytime. The stability of humidity and the low load of solar radiation are key factor of the occurrence according to our results. The shaded refugees (plausibly the cracks of the rocks) can provide escape routes when the in the weather gets too dry and hot in summer.

The successful collecting sites were near to the shaded guarry wall and the stone blocks, while the less-successful 'q' site was in a much more sun-lit environment further away from the wall. In the Judean desert, Phlebotomus sergenti Parrot (1917) individuals were also trapped outside covered caves, probably arriving from smaller, concealed cracks (Moncaz et al., 2012). The hot and dry guarry yard of the Nagyharsány Sculpture Park provided similar microclimatic conditions in summer like the open part of a hot desert. In contrast, the cracks of the rocky ledges created similar mild environment like the shaded fissures of the boulders in the Judean Desert. It can explain why stone walls and rock piles are frequent breeding habitats of Phlebotomine sandflies (Moncaz et al., 2014. The moderately elevated temperature conditions alone are not disadvantageous for sandfly larvae. The tolerable temperature range of Ph. neglectus larvae is about -4 to 30°C (Lindgren et al., 2006). Both the host seeking behavior of sandfly species and *Leishmania* development in the sand fly midgut are significantly affected by temperature (Benkova and Volf, 2007). It is known that the number of collectable adult sand flies positively correlate with the mean temperature (Sangiorgi et al., 2012). For most Phlebotomus species, the optimum temperature is between 24 to 28° C. (Volf and Volfova, 2011). Based on this fact, it may seems that the daily mean air temperatures were sub-optimal in each collecting day. However, this is true only for the average air temperature. Both in the successful and non-successful collecting sites, the maximum temperatures of each sites exceeded this value in 28 July 2014. It is very plausible that the surface temperature of rocks was continuously within the optimum range.

In late morning and the first half of afternoon, the shaded south part of the quarry was cooler than the central and north part which received direct sunshine in this period. In contrast, from the evening to night hours, the south points were warmer than the north points which cannot be explained by topographic differences since the recultivated and filled pit is nearly flat. It can be the result of heat-mirror effect as the heat radiation of the north quarry wall project to the south wall. Although the air temperatures also showed characteristic spatial differences, it is plausible that similar, but even more notable differences existed between the surface temperatures of the north and south sites. The residual heat could strongly influence of near-ground humidity or the CO₂ emanation and the scent of vegetation. It cannot be excluded that local CO₂ emanation of the vegetation can really influence the diurnal activity of sandflies. According to Beavers *et al.* (2004), CO₂ is an effective attractant for female

Ph. papatasi individuals and it is also known that CO₂-baited CDC light traps are attractive for *Phlebotomus* sandflies (Kasili *et al.*, 2009). The close relative *Lutzomyia* sandflies are also sensible to carbon-dioxide (Pinto *et al.*, 2001). It should be noted, that more dense vegetation can be found at the most successful trapping sites in the quarry where the shaded and more humid conditions of the south quarry wall allow the survive of flowering ash trees. Near-ground humidity were not measured, but it can be assumed that due to the transpiration of the ligneous vegetation it could increase in the evening hours and remain higher in the night hours compared to the bare north quarry wall or even the grassy central pit area where dense ligneous vegetation doesn't occur. On the other hand, the shaded environment of the south wall during mid-day and the afternoon hours plausibly can prevent the drastic decrease of the near-ground humidity which can be very beneficial for both larvae and imagoes. The preferred relative air humidity of *Ph. neglectus* adults is about 60-80% (Lindgren *et al.*, 2006) which condition can persist in the cracks during the hottest hours of the day.

The limitations of our study should also be noted. First, while trappings were conducted in some consecutive years, the measurement of air temperature and the daytime patterns of solar radiation were measured only on the sample day. On the other hand, the number of trapping sites was less than the number of measuring sites. Despite this bias, the results of this study provide valuable information about the microhabitat preference of Ph. neglectus since: i) turn of July and August is the hottest and driest period in Hungary which represents the most unfavorable combination of solar radiation conditions and ambient temperature for such a humidity and moisture-demanding species as Ph. neglectus. Although measurements were performed only on one day, due to the dry atmospheric conditions, solar radiation patterns of this day can represent sufficiently the light conditions of the main part of summer since the solar radiation conditions show minor changes due to relative proximity of the summer solstice. ii) The number and the location of trapping and measuring sites were different. The numerous measuring sites were involved into the study for the correct interpolation of the measured values. It is worth pointing out that in the proximity of the partly shaded and vegetation-rich Ph. neglectus habitats the different mosquito traps did not collected sandfly individuals.

Our results are in accordance with the findings of Howlett (1913) who concluded that breeding sites of *Phlebotomus* species are restricted to shaded, light protected habitats. It is plausible that *Ph. neglectus* prefers shaded environments to escape from heat and desiccation in the dry season. We observed rich moss vegetation growing under the canopy of the flowering ash on the large stone blocks of the most successful trapping site which can be a similarly humid environment as the cracks of water wells, a preferred habitat of sandfly species (Chaniotis and Tselentis, 1996). Cracks and cavities of thicker stone walls could provide excellent inner conditions for *Phlebotomus* species despite the unfavorable outer solar radiation and temperature climate conditions (Surendran *et al.*, 2007; King, 1914). Although trapping activity were performed in three different days, the spatial pattern of the occurrence of sandfly individuals remained consequently very similar which refers to the stability of the habitat site patterns.

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