



Research Article Seasonal abundance of Tabanidae (Diptera) on a farm in southern France.

The primary aim of the current study was to monitor the phenology of the Tabanidae on a farm in southern France, near Montélimar.

The phenology or seasonal activity patterns of hematophagous insects such as the Tabanidae could help to prevent stress and pathogen transmission during peaks of abundance through the implementation of proper control strategies (Altunsoy and Kılıç, 2012).

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# Introduction

The Tabanidae family has considerable significance in medical and veterinary entomology. The family's importance is associated with both the potential transmission of pathogens and the nuisance resulting from painful bites, with indirect secondary infections, myiasis, anemia, stress and allergic responses (Chvala et al., 1972; Krinsky, 1976; Baldacchino et al., 2014a).

Because of the bloodsucking feeding behavior of female tabanids, many worldwide studies have been published on this family (Cameron, 1926; Maccreary, 1940; Blickle, 1955; Leclercq, 1966; Bosler and Hansens, 1974; Kniepert, 1980; Andreeva, 1989; Krcmar et al., 2005; Krcmar 2006; Andreeva et al., 2009; Altunsoy and Kılıç 2010; 2012). Dense tabanid populations can have an important economic impact

on outdoor activities, tourism and livestock breeding and other animal linked activities, including agriculture. For example, pastured cattle may suffer severely from heavy attacks of tabanids causing loss in weight gain or milk production (Goodwin and Drees, 1996; Desquesnes, 2004; Baldacchino et al., 2014a).

The Tabanidae family consists globally of about 4,400 known species of medium-to-very-large biting flies. It represents about 650 species in the Palearctic region and 83 species in France (Chvala et al., 1972).

But despite the importance of livestock husbandry in France, there are a few studies on the distribution and seasonal activity of tabanid species (Baldacchino et al., 2014b).



# Materials and Methods

## Study area

The study was performed in a farm near the village of Allan (44°30'30,03"N, 4°49'00,63"E, at 242 m elevation). This farm was chosen because it was reputed to have a large abundance of tabanids during the summer period.

The farm contained 3 Ha of pasture and was isolated in the middle of a forest, with many humid areas. There were very few domestic animals on the farm during the study period consisted of: horses (2), donkeys (2), cows (1), pigs (2) and several geese and fowl.

Wild animals such as wild boars, roe deer, hares and foxes were reputedly abundant.

### **Trapping**

After an earlier comparison of the efficiency of different traps (H-Trap, Nzi trap, Vavoua trap, and sticky blue screen) on this farm, it was decided to use H-trap and TaonX for the current study. The H-Trap gave the best results in terms of attractivity (number of captured tabanids) and specificity (very few non-target fauna) (Ferjani, 2017).

The Bite-Lite® H-Trap (Fig. 1) is a conical, plastic-hooded trap attached to a single-legged frame with an anti-rotation anchor. Flies are attracted to a black ball (diameter 50 cm) suspended beneath the plastic hood, which guides the flies upwards into

a centrally located plastic collection device (Kline et al., 2018). The 5 H-Trap was used, in addition to two Kerbl® TaonX already in use by the farm owners. The Bite-Lite® and Kerbl® units are similar as only the fixation of the plastic collection cage is different.



Fig. 1 H-Trap (Photo G. Duvallet)

The placement of the traps is indicated in Fig. 2. The distances between traps were in the range 30 - 60 m and they were all placed in very similar environments on the meadow and near the limit of the forest.



Fig. 2 A map showing locations of Traps on the farm site, where AH-1—AH-5 = H-Traps and KH-1 and KH-2 = Kerbl® TaonX (Image from Google Earth)

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## Experimental design

Previous studies on seasonal abundance patterns in Europe indicated that the first species emerge in the second half of May and persist until mid-September (Chvala et al., 1972; Krcmar 1999; 2006; Krcmar et al., 2005). Consequently, the current study was conducted from early May until mid-September 2017.

Traps were activated on 3 May 2017 and the last collection of tabanids was on 17 September 2017. The collection devices on top of each unit were filled with about 100 mL of tap water mixed with 3 ml of liquid soap.

This mixture was saturated with NaC1 to limit the fermentation of captured insects. Devices were emptied about every 7 d and the insects from each trap were transferred into flasks containing 70% ethanol. Specimens were identified to known species using the keys from Chvala et al. (1972) and Leclercq (1966).

### Climatic data

Daily climatic data were collected from the Montélimar weather station, situated 8 km from the farm. These data were: minimum, average and maximum temperatures, minimum, average and maximum relative humidity, daily precipitation and insolation duration.

### Statistical analysis

Analyses were done using the R programming language for statistical computing and graphics (R Core Team, 2015) within

R Studio version 3.5.1, an integrated development environment for R (RStudio Team, 2015). With these non-parametric data, a Kruskal-Wallis test was done to compare the tabanid trapping scores for the traps.

After that, a multiple comparison test was done to identify which traps were different using the 'kruskalmc' function of the 'pgirmess' package in R. The Shannon-Wiener index was calculated for every week (using the 'pgirmess' package and the 'Shannon' function in R).

#### Results

In total, 54,618 specimens belonging to the family Tabanidae, 3 subfamilies, the Chrysopsinae, Pangoniinae and Tabaninae, 6 genera and at least 19 species were collected during the 21 wk of observation (3 May - 17 September 2017). The number of species was probably higher, because although all the specimens of the genus Haematopota belonged to the group italica, there were probably several different species in the group, not yet separated at this stage.

The weekly number of the Tabanidae captured in the 7 units is indicated on *Fig. 3*. There were two peaks of abundance, one around the end of May with 2,906 tabanids in 1 wk, and the other in the second half of August with 14,834 tabanids in 1 wk. Only 3 species were captured during the first week (3 May), while 15 different species were captured during each of the weeks 5 July, 12 July and 6 August (*Fig. 3*).

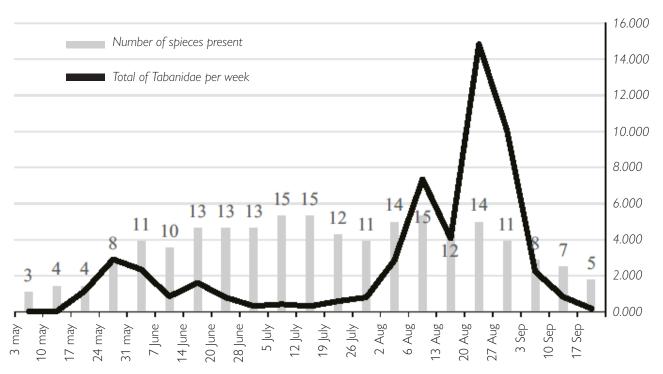


Fig. 3 Weekly numbers of species and specimens of Tabanidae captured using 7 units on the study site during 2017 season of tabanid activity

# Results

Table 1 gives the results per species and per week. Tabanus was the most important genus with 14 different species, but the genus Haematopota was the most abundant with 33,116 specimens (61% of captures) followed by T. glaucopis (11%), T. nemoralis (8%), T. quatuornotatus (4%), T. exclusus (4%) and T. bromius (3%) as shown in *Fig. 4*. All the other taxa each represented less than 2% of captures.

Table 1 also shows the seasonal activity of all species. Precocious species such as T. nemoralis, T. quatuornotatus, T. cordiger, T. autumnalis and Haematopota sp. were already present by 3 - 10 May. Haematopota sp. was present throughout the season with a large peak of abundance at the end of August. Tabanus nemoralis and T. quatuornotatus were present during a shorter period with a peak of abundance at the end of May; they disappeared at the end of June.

A few rare specimens of T. nemoralis were captured again in August. Other species such as T. exclusus, Pangonius micans, T. glaucopis, Atylotus loewianus, T. eggeri and T. paradoxus appeared later, at the end of June. Tabanus exclusus was present 20 June - 3 September, with a peak of abundance in early August. Only six specimens of Pangonius micans were captured in July. Three specimens of T. glaucopis were captured in early July but most were captured in August and they were present until the end of the capturing period. Atylotus loewianus were present 5 July - 27 August, with a peak of abundance in early August. Tabanus eggeri and T. paradoxus were present from early July to early September, with a peak of abundance in early August. T. paradoxus was most active for about 1.5 hr around dusk. This could be confirmed by a specific study on the daily activity of these insects.

The remaining species T. tergestinus, T. bromius, Dasyrhamphis ater, T. bifarius, T. unifasciatus, Chrysops viduatus, T. rectus and T. regularis were captured from the end of May to early June until the end of the capturing period. Tabanus bromius and T. tergestinus had a peak of abundance in June for T. tergestinus and in August for T. bromius. Dasyrhamphis ater. and T. bifarius were present from the end of May to the end of June and into early July, with peak abundance in early June.

Tabanus unifasciatus and T. regularis were present from the end of May and mid-June respectively until the end of August with peak abundance in early August. Isolated specimens were captured in September. Only 17 specimens of C. viduatus were captured between June and August. T. rectus specimens were captured in relatively low numbers between June and August, with a small peak of abundance in early July.

Fig. 4 shows the population dynamics of the six most abundant species throughout the study. An indication of the efficiency of each of the seven units is given in Fig. 5. Two traps produced different results: KH1 with 2%, and AH4 with 24% of all specimens captured. The other traps captured between 12% and 17% of all the specimens and there were no significant differences among them. However, traps AH1 and KH2 were significantly different from trap AH4.

Chi-square tests showed no differences in the composition of captures among the 7 traps. As mentioned earlier, the environments of the different traps were very similar, but the presence of the captured specimens was variable. Furthermore, up to this stage there was no indication on larval development sites in this area.

Species	May				June				July				August				September				Total		
	3	10	17	24	31	7	14	20	28	5	12	19	26		2	6	13	20	27	3	10	17	
Tabanus nemoralis	5	20	402	1948	1413	274	211	13	1	0	0	0	0		0	21	0	41	0	0	0	0	4349
T. quatuornotatus	7	8	668	846	581	97	34	0	0	0	0	0	0		0	0	0	0	0	0	0	0	2241
T. cordiger	1	0	0	2	13	6	19	9	1	2	1	0	0	4	10	48	9	19	3	5	4	1	183
T. autumnalis	0	3	9	17	27	11	24	1	1	4	2	0	0		1	1	0	3	0	0	0	0	104
Haematopota gr. italica	0	2	90	81	161	166	246	226	51	30	27	132	369	12	26	4129	2875	11796	8644	1954	745	166	33116
T. tergestinus	0	0	0	1	1	4	231	297	100	210	55	16	84	1	58	85	8	4	13	1	1	0	1269
T. bromius	0	0	0	1	17	48	190	68	55	47	61	108	112	2	77	493	96	110	12	8	10	2	1715
Dasyrhamphis ater	0	0	0	10	52	40	50	10	1	0	0	0	0		0	0	0	0	0	0	0	0	163
T. bifarius	0	0	0	0	70	161	465	51	2	2	1	1	0		0	0	0	0	3	0	0	0	756
T. unifasciatus	0	0	0	0	5	42	108	61	50	25	29	48	25	1	88	119	56	12	21	0	0	1	790
Chrysops viduatus	0	0	0	0	1	0	6	1	3	1	1	0	1		1	1	1	0	0	0	0	0	17
T. rectus	0	0	0	0	0	0	15	37	40	54	49	55	8		2	9	0	1	0	0	0	0	280
T. regularis	0	0	0	0	0	0	12	25	10	10	20	38	31		8	87	16	22	13	0	1	0	343
T. exclusus	0	0	0	0	0	0	0	1	9	26	48	150	102	6	13	922	109	77	4	7	0	0	2068
P. micans	0	0	0	0	0	0	0	0	0	4	1	1	0		0	0	0	0	0	0	0	0	6
T. glaucopis	0	0	0	0	0	0	0	0	0	3	0	0	0	7	76	1019	801	2630	1302	255	66	13	6165
Atylotus loewianus	0	0	0	0	0	0	0	0	0	2	2	7	9	1	18	186	55	72	20	0	0	0	471
T. eggeri	0	0	0	0	0	0	0	0	0	3	15	20	6	2	2	28	4	2	0	1	0	0	101
T. paradoxus	0	0	0	0	0	0	0	0	0	0	3	18	57	,	2	208	47	45	21	9	1	0	481
Total	13	33	1169	2906	2341	849	1611	800	324	423	315	594	804	28	62	7356	4077	14834	10056	2240	828	183	54618

Table 1 Phenology and abundance: Number of specimens captured per species and per week using 7 units on the study site

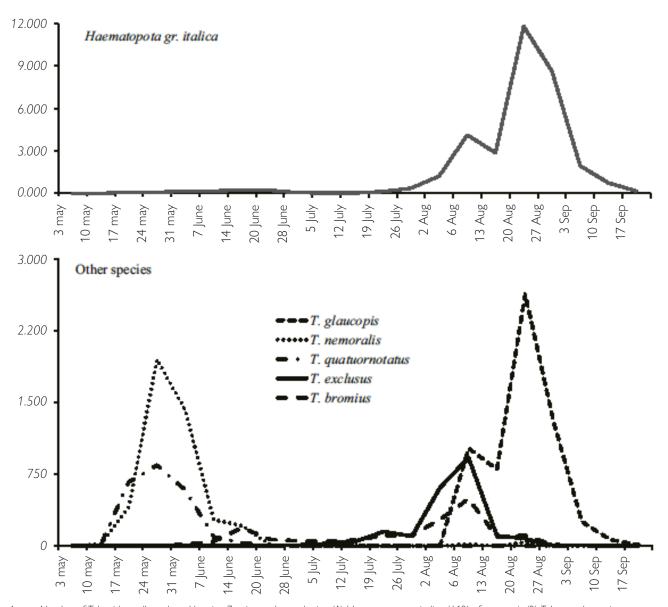


Fig. 4 Number of Tabanidae collected weekly using 7 units on the study site: (A) Haematopota gr. italica (61% of captures); (B) Tabanus glaucopis (11% of captures), T. nemoralis (8% of captures), T. quatuomotatus (4% of captures), T. exclusus (4% of captures) and T. bromius (3% of captures)

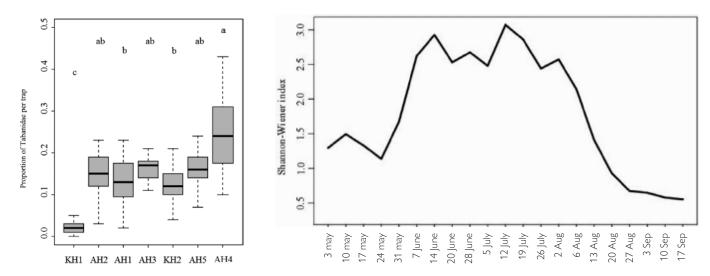


Fig. 5 Proportion of Tabanidae captured in each trap during the study, where different lowercase letters above a column indicate a significant (p < 0.05) difference and error bars indicate  $\pm$  SD.

Fig. 6 Shannon-Wiener index calculated for every week during the study

Fig. 6 shows the evolution of the Shannon-Wiener index throughout the season. Values were in the range 0.5—3 and provide an indication of the weekly numbers of species present and the distribution of specimens among the species. The highest values for index indicated that the area was dominated by only one species (Haematopota italica).

The data collected at the weather station for the period of the study were typical of the northern part of the Mediterranean area with some precipitation at the beginning of the spring period followed by very little rain during the rest of the season. Temperatures, linked to insolation, were high from June to August. However, there was no significative correlation between the climatic data and the abundance of different species.

#### Discussion

The seasonal activity of the Tabanidae on a farm in southern France was monitored during the 2017 fly season. The results suggested that this farm is a hot spot of biodiversity and abundance for the Tabanidae, at least in Europe. In the Lazio region, Central Italy, De Libertado et al. (2019) captured 18,701 tabanids belonging to 39 species over several sites, with 26 of these species captured on a single site.

The species richness and abundance in the current study area could be explained by several factors such as the inclusion of the farm in the middle of a forest with a long ecotone between forest and meadows, the presence of several springs and humid areas favorable to larval development around the farm, the presence of wild animals in the forest (such as roe deer and wild boar) in addition to domestic animals, a Mediterranean climate with mild winter conditions and hot summers and a complete absence of the use of insecticides.

In their survey in the Pyrenees mountains, Baldacchino et al. (2014b) captured 19 species over the period 2011-2012. In Korea, Suh et al. (2015) captured 20 different species during their survey, but only 9 at the same time. In the eastern part of Algeria, Zeghouma et al. (2018) captured 15 different species during the entire fly season in three different spots. In their study of the Occidental Rif in Morocco, El Haouarri et al. (2014) identified 125 different species of the Tabanidae in Morocco, but only a maximum of 7 species for any single site. In their review of horse flies in Europe, Chvala et al. (1972) indicated the number of species known in each country and clearly there was a gradient of abundance from the north to the south.

Only H-Traps were used in the current survey where they were efficient in terms of the high number of the Tabanidae captured and the low number of nontarget fauna (data not shown). Nevertheless, it is worth considering whether or not their efficiency was the same for all species.

Clearly, they were very efficient for the capture of Haematopota species. In contrast, very few Chrysops viduatus were captured, although numerous specimens were observed feeding on the horses backs on many occasions during the field work. The most frequently used trap for the Tabanidae has until now been the

Nzi trap (Mihok, 2002). Baldacchino et al. (2014b) have indicated that Nzi traps seemed less effective for Chrysops spp. and Haematopota spp which suggests there is no universal trapping system for insects such as the Tabanidae and the best way to undertake inventory surveys would be to use different traps at the same time. The non-hematophagous species Pangonius micans was captured on only three occasions in July. Considered as a South European species (Chvala et al., 1972) active from May until August, this species is usually seen sucking flowers and so was probably not attracted to the H-Trap.

The current study was primarily designed to investigate the seasonal activity of the Tabanidae species. It can be clearly seen that some species were only active during certain months, such as Tabanus quatuornotatus, which was present from the beginning of May to mid-June, with a peak of abundance on 24 May. This was in accordance with the observations of Chvala et al. (1972) who considered this as a typical spring species, usually the first species which is on the wing in Central Europe.

This was also the case for D. ater, present only at the end of May and in June. This period of activity was shorter than the period of May until August indicated by Chvala et al. (1972) but they were considering a larger area of distribution. The same observation was made for T. bifarius, a common species in the Mediterranean region, usually active from May to August, but seen only from the end of May until the beginning of July in the current study.

Tabanus unifasciatus, known also as a South Europe species was captured throughout the period of activity indicated by Chvala et al. (1972), from June to the end of August. The species T. regularis, T. rectus and Chrysops viduatus were active from June to August, as indicated by Chvala et al. (1972). T. glaucopis also was only active during a short period in August and September, as in the Pyrenees mountains (Baldacchino et al., 2014b).

This species was considered by Chvala et al. (1972) as an Eurasiatic species with a period of activity from June to September. While T. bromius was reported to be active only during a short period (late July—early August) in the Pyrenees mountains (Baldacchino et al., 2014b), this species was present for a much longer period (the end of May to the end of September) in the current study area. This was clearly linked with the higher elevation of the sites studied by Baldacchino et al. (2014b). All 33,116 specimens of the genus Haematopota captured in the current survey belonged to the group italica, with a cylindrical and rather slender antennal segment 1, at least four times as long as deep (Chvala et al., 1972). The systematics of the genus Haematopota are not yet completely established and a lot of work must still be done to separate this group into different species.

The Haematopota specimens were present throughout the season from May to September, with a huge peak of abundance in the week 14–20 August, with 11,796 specimens in 1 wk for the seven traps. During this period of the year, those tabanid

species are a major pest not only for domestic animals, but also for the owners of the farm and the tourists trying to walk across this area.

Overall, the seasonal activity in the current survey enabled the identification of some precocious species (T. nemoralis, T. quatuornotatus, T. cordiger and T. autumnalis), some intermediate species active during a long period of the fly season (Haematopota gr. italica, T. tergestinus, T. bromius, T. regularis) and some late species active only from July (T. glaucopis, Atylotus loewianus, T. eggeri, T. paradoxus).

A future study of the larval development sites of these different species would provide additional ecological data for better understanding and control of these significant pests.

#### **Conflict of Interest**

The authors declare that there are no conflicts of interest.

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