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SEASONAL AND GEOGRAPHICAL DISTRIBUTION OF *CULICOIDES IMICOLA* KIEFFER (DIPTERA: CERATOPOGONIDAE) IN SOUTHWESTERN SPAIN

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ABSTRACT. *Culicoides imicola* Kieffer adults were collected in light traps weekly between August 1990 and October 1991. The species was collected at all 62 sites located in 5 provinces of the Autonomous Region of Andalucía (Spain). *Culicoides imicola* represented 31.7% of the total collection of *Culicoides*. The highest numbers of *C. imicola* were collected from September through November 1990 and September and October 1991. The lowest numbers were collected from December 1990 through April 1991. The presence or absence of *C. imicola* was related to the mean monthly minimum and maximum air temperature. Greatest numbers of *C. imicola* were collected at daily minimum and maximum temperatures of 18°C and 38°C, respectively. The relevance of this information to previous outbreaks of African horse sickness is discussed.

INTRODUCTION

African horse sickness (AHS) virus serotype 4 was isolated in 1989 from pools of *Culicoides imicola* Kieffer, and from pools of *Culicoides pulicaris* L. and *Culicoides obsoletus* Meigen in Andalucía, Spain (Mellor et al. 1990). Because of these virus isolations, it was deemed important to further delineate the distribution of the vectors of AHS virus in Spain.

Culicoides imicola has been reported in the Mediterranean region from Egypt (Macfie 1943), Morocco (Kremer et al. 1971), Algeria (Szadziewski 1984), Spain (Mellor et al. 1983), Portugal (Mellor et al. 1985), Cyprus (Jennings et al. 1983), western Turkey (Jennings et al. 1983), Lesbos (Boorman and Wilkinson 1983), Rhodes (Mellor et al. 1984), and Israel (Callot et al. 1969). The species has not been recorded from surveys of Italy, Greece, or France (Mellor et al. 1984); Tunisia (Chaker 1981); the Balearic Islands (Boorman et al. 1985); or the islands of Sardinia, Corsica, Sicily, Malta, or Crete (Boorman et al. 1985).

This work reports the results of surveillance for *C. imicola* in the provinces of the Autonomous Region of Andalucía where cases of AHS had occurred.

MATERIALS AND METHODS

The study was conducted from August 1990 through October 1991. Light traps were established at 62 individual farms: 11 in Cádiz Province, 8 in Córdoba Province, 11 in Huelva Province, 13 in Málaga Province, and 19 in Sevilla Province (Table 1). The farms where *Culicoides* were collected were located between 36°12′N and 38°22′N latitude and 4°6′W and 7°35′W longitude. Some of the farms were located in the vicinity of meteorological stations operated by the Regional Meteorological Services of Sevilla and Málaga. These stations provided daily precipitation data, and some provided both daily data on precipitation and/or maximum–minimum temperatures.

Adult *Culicoides* were collected in baffle-type light traps with a 60-W 220-V bulb (Lillie et al. 1979). The traps were located in outdoor areas in the vicinity of domesticated animals (e.g., cattle, sheep, goats, horses, dogs, and poultry). Although it has been demonstrated that these animals are not equally attractive to *C. imicola* (Braverman et al. 1971, Braverman and Chizov-Ginzburg 1996), no attempt was made to standardize the potential hosts at a collection site, and an inventory of the animals from each site is not available. All traps were suspended at a height of 1.5 m, from either trees or man-made structures. The insects were collected in 0.5-liter flasks containing 17.5% ethanol and 15% ethylene glycol (to reduce evaporation) in water. Collections were later transferred to 70% ethanol for preservation.

Traps were operated from approximately 1 h before sunset to 1 h after sunrise for 2 consecutive nights per week. Occasionally, traps were operated for only one night in a given week, and a few times they were operated for more than 2 consecutive nights. An individual collection, therefore, might represent the insects collected on from one to several nights. On rare occasions traps malfunctioned or were vandalized and collections from those traps are missing.

All *Culicoides* spp. were initially sorted with the aid of a stereo-zoom microscope (2.5× to 64× magnification). *Culicoides imicola* was identified by its characteristic wing pattern (Boorman 1989; Boorman, personal communication 1993). Other *Culicoides* species were identified but will be reported elsewhere.

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Table 1. Geographical location of the 62 farms at which light traps were established in 5 provinces of Andalucía, August 1990 through October 1991, indicating where temperature (T) or precipitation (P) data were available.

City	Latitude (N)/longitude (W)
Cádiz Province	
S. Fernando	36°17′/6°10′
Sanlúcar (P)	36°47′/6°17′
Trebujena (P)	36°52′/6°11′
Rota (TP)	36°17′/6°17′
Olvera (TP)	36°55′/5°15′
Algodonales (P)	36°53′/5°25′
Jerez (TP)	36°43′/6°03′
Medina (TP)	36°27′/5°55′
Barbate	36°12′/5°56′
Castellar	36°17′/5°25′
San Roque (P)	36°13′/5°23′
Córdoba Province	
Córdoba (TP)	37°15′/4°48′
Posadas (P)	37°49′/5°08′
Peñarroya (TP)	38°18′/5°13′
Pozoblanco	38°22′/4°48′
Cardeña (TP)	38°12′/4°17′
Montoro (TP)	38°01′/4°21′
Baena (P)	37°37′/4°17′
Lucena (TP)	37°24′/4°23′
Huelva Province	
Aracena (TP)	37°52′/6°33′
Aracena (TP)	37°51′/6°17′
Aroche (TP)	37°58′/6°58′
Rosal	37°58′/7°35′
Cabeza (TP)	37°47′/7°08′
Andévalo	37°42′/7°10′
Valverde (TP)	37°34′/6°53′
Niebla (TP)	37°23′/6°41′
Huelva (TP)	37°20′/6°54′
Huelva (TP)	37°15′/6°55′
Málaga Province	
Gaucín (TP)	36°33′/5°17′
Mijas (TP)	36°36′/4°37′
Pizarra	36°45′/4°40′
Álora (P)	36°49′/4°45′
Rincón (P)	36°43′/4°15′
Antequera (TP)	37°03′/4°34′
Málaga (TP)	36°32′/4°26′
Marbella (TP)	36°30′/4°53′
Casabermeja (TP)	36°53′/4°27′
Teba (TP)	36°57′/4°52′
Vélez (TP)	36°47′/4°06′
Alhaurín (TP)	36°40′/4°34′
Ronda (TP)	36°44′/5°09′
Sevilla Province	
Alcalá (TP)	37°20′/5°50′
Sevilla (TP)	37°25′/6°00′
Castilleja	37°25′/6°05′
Alcolea	37°37′/5°39′
Puebla (TP)	37°15′/6°05′
Villanueva (P)	37°35′/5°40′
Constatina	37°50′/5°35′
Lora (TP)	37°32′/5°37′
Peñaflor (TP)	37°40′/5°17′
Osuna (TP)	37°19′/5°05′
Osuna (TP)	37°18′/5°10′
Utrera (TP)	37°10′/5°44′
Utrera (TP)	37°10′/5°46′
Ecija (P)	37°35′/5°05′
Ecija (P)	37°30′/5°06′
Ecija (P)	37°45′/5°09′
La Campana	37°34′/5°24′
Lora	37°38′/5°33′
Fuentes (TP)	37°23′/5°20′

RESULTS AND DISCUSSION

Culicoides imicola was the predominant species of *Culicoides* collected in the study, and was collected at least once at all sample sites listed in Table 1. Of the 177,344 adult *Culicoides* in 3,109 individual trap collections, 56,254, or 32%, were *C. imicola*. *Culicoides imicola* was found in 30% of all trap collections, and comprised 42% of trap collections with *Culicoides*.

In studies in South Africa and Portugal *C. imicola* accounted for up to 97.4% (Nevill 1971) and 80% (Capela et al. 1993), respectively, of the total *Culicoides* collected. In Nigeria, *C. imicola* represented 37.6% (Dipeolu and Sellers 1977) and 26% (Herniman et al. 1983), and in Lesbos (Boorman and Wilkinson 1983) the species represented 27% of the total *Culicoides*. *Culicoides imicola* was also the predominant *Culicoides* species collected in studies in Kenya (Davies and Walker 1974), Turkey (Burgu et al. 1992), and Israel (Braverman et al. 1985, Braverman and Linley 1993).

The mean number of *C. imicola* per trap night and the mean number of all species of *Culicoides* combined per trap night were calculated for each of the months of the study and are presented in Table 2. Although fluctuations in seasonality may occur, in the present study, the months with the highest numbers of *C. imicola* per trap night were September, October, and November in 1990 and September and October in 1991, when the study was terminated. From September through November in 1990, *C. imicola* outnumbered all other species of *Culicoides* combined. *Culicoides imicola* was also found to be the predominant species in September and October in Portugal (Capela et al. 1993) and Turkey (Burgu et al. 1992). In warmer climates, such as those of Israel (Braverman et al. 1985) and Nigeria (Herniman et al. 1983) greater numbers of adult *C. imicola* occur earlier in the year (i.e., in late August or September). Braverman et al. (1985), in Israel, reported that *C. imicola* populations began to increase in July, and that these high populations persisted through November, and did not decrease during the 2nd half of November as we found in Andalucía.

Table 2. Mean numbers of *Culicoides imicola* and all *Culicoides* species per trap night, and monthly mean minimum and maximum temperatures and precipitation at the locations with meteorological stations.

Month	*C. imicola*		All *Culicoides*		Mean temperature		Mean precipitation (mm)
	Mean number	SE	Mean number	SE	Minimum (°C)	Maximum (°C)	
1990							
Aug.	10.4	3.9	30.9	5.4	19.3	33.4	0.04
Sept.	39.3	19.5	59.3	20.0	18.1	30.7	0.36
Oct.	43.6	23.5	56.3	23.9	13.0	22.0	3.26
Nov.	27.9	23.3	40.4	24.3	8.1	17.5	2.18
Dec.	0.5	0.2	8.2	2.1	5.3	13.6	1.27
1991							
Jan.	0.1	<0.1	2.6	0.6	4.2	14.0	0.59
Feb.	<0.1	<0.1	2.6	0.6	4.2	13.5	4.12
March	0.1	<0.1	21.4	4.3	8.2	17.0	4.10
April	0.7	0.3	23.3	3.1	8.1	16.3	1.48
May	1.2	0.5	50.4	10.1	10.9	24.4	0.06
June	1.2	0.4	76.0	26.7	15.9	30.1	0.34
July	3.8	0.9	41.8	7.7	18.8	34.3	0.04
Aug.	3.2	0.7	21.0	3.7	19.3	34.7	0.02
Sept.	10.5	3.7	31.3	10.0	17.3	30.4	1.30
Oct.	14.8	7.8	25.5	8.8	10.8	20.9	3.91

Culicoides imicola adults were collected in very low numbers from December through August and were absent from most areas between January and March. The insects were present only in Cádiz and Sevilla during January and in Cádiz during February. These were the coldest months of the year in these locations, with mean monthly minimum temperatures of 4.2°C, and mean monthly maximum temperatures of 14°C and 13.5°C, respectively (Table 2). Similarly, these insects were not present in a study in Turkey from December through February (Burgu et al. 1992).

In these study areas, July and August were the hottest months of the year, with mean minimum temperatures of 18.8°C and 19.3°C and mean maximum temperatures between 34.3°C and 34.7°C, respectively. Peak numbers of *C. imicola* appeared following the hottest months, but numbers were reduced sharply as soon as mean maximum temperatures fell below 20°C. This finding is in agreement with that of Nevill (1971) who reported that in South Africa low temperatures during the winter months slowed development of *Culicoides*.

Although *Culicoides imicola* was collected at all 62 trap locations, the mean number per trap night varied from 0.01 to 455. Four traps collected more than 89% of the total *C. imicola*. These traps were located in the province of Cádiz at the Sotogrande site in San Roque, in the province of Huelva at the Belleza Jara site in Aroche, and in the province of Sevilla at the Valdevacas and Dehesa Frias sites in Villanueva Río and Constatina, respectively (Table 3). In general, high numbers were collected during September and October at these trap sites. Additional population peaks occurred in Sotogrande in April and May and in Dehesa Frias in July 1991. The presence of *C. imicola* at all the sites, and throughout the year, suggests that *C. imicola* is not a temporary species in Spain as was previously suggested by Mellor et al. (1985).

Details on the suitability of all the collection sites as habitats for *C. imicola* are not available. A large horse population was reported in the area of the Sotogrande site at the time of the survey and may have attracted adults and served as hosts as well as contributed to the larval habitat. The presence of large numbers of *C. imicola* in Sotogrande during November 1990 (Table 3) was due to collections made during the first half of the month when mean minimum temperatures were greater than 10°C and mean maximum temperatures were greater than 20°C. The presence of *C. imicola* adults throughout the year in some areas appeared to be associated with milder temperatures. According to Nevill (1971), *Culicoides* adults are occasionally active during winter nights, and in some years remain active throughout the winter when daytime temperatures are high enough to allow continued development.

Both in our study and elsewhere (Dipeolu and Orunginade 1977, Herniman et al. 1983, Braverman and Linley 1988), high numbers of *C. imicola* were associated with the end of summer. Indications also have been found that high numbers of *C. imicola* follow periods of rain (Nevill 1971, Dipeolu and Sellers 1977). Rain appeared to have no effect in the present study, whereas in South Africa rains at the end of summer produced large populations of *C. imicola* (Nevill 1971, Venter and Sweetman 1989).

Braverman and Galun (1973) suggested that during late summer, the time when peak numbers of

Table 3. Mean number of *Culicoides imicola* per trap night at the 4 locations in Andalucía responsible for approximately 90% of the collection, August 1990 through October 1991; Cádiz Province, San Roque, Sotogrande site; Huelva Province, Aroche, Belleza Jara site; Sevilla Province, Villaneuva Río, Valdevacas site; and Sevilla, Province, Constatina, Dehesa Frias site. Meteorological data were available only at Sotogrande and Belleza Jara.

	Sotogrande			Belleza Jara			Valdevacas	Dehesa Frias
		Mean temperature (°C)			Mean temperature (°C)			
Month	No. *C. imicola*	Minimum	Maximum	No. *C. imicola*	Minimum	Maximum	No. *C. imicola*	No. *C. imicola*
1990								
Aug.	219	18.7	32.0	2	20.6	36.2	24	24
Sept.	1679	18.7	30.6	4	—	—	69	71
Oct.	1993	13.6	24.3	7	11.8	20.4	26	27
Nov.	1296	8.5	19.7	33	—	—	6	6
Dec.	0	8.4	15.9	0	—	—	6	5
1991								
Jan.	2	5.1	16.2	0	5.0	11.4	<1	<1
Feb.	<1	4.7	15.2	0	3.2	11.6	0	0
March	3	9.0	18.2	0	6.8	12.1	0	<1
April	34	8.8	18.2	<1	8.6	14.5	2	1
May	52	11.4	22.2	1	11.5	21.0	2	9
June	7	15.3	26.8	<1	12.0	33.1	11	13
July	17	17.4	31.6	35	18.8	36.3	17	47
Aug.	57	18.4	32.4	28	18.5	35.1	8	23
Sept.	245	17.6	29.8	178	—	—	23	21
Oct.	399	14.5	26.7	173	11.4	21.5	37	1

Culicoides were found, temperatures are favorable for breeding and numerous, small breeding sites that are not dependent on rainwater are available. In Israel, as in Spain, the rainy season is restricted to the winter months or cold season. Thus, increased rainfall has no immediate effect on the size of the *C. imicola* population. According to Braverman and Galun (1973) if the winter season is prolonged and rainy, more puddles persist to serve as breeding places of the spring generation of *Culicoides,* which in turn may result in a larger autumn population. In the present study, a small peak of adult activity occurred in the spring and a second, larger one occurred in the fall, similar to that recorded by Braverman and Galun (1973) in Israel, and Nevill (1971) in South Africa.

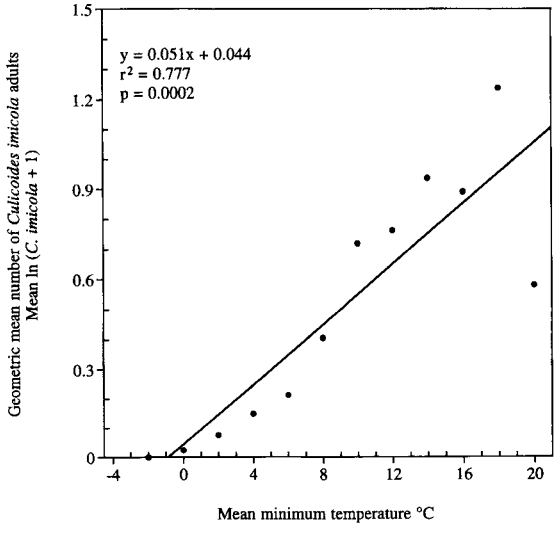

Fig. 1. Geometric mean number of *Culicoides imicola* adults per trap night versus mean minimum daily temperature.

The data presented here support the hypothesis that *C. imicola* adults tend to be collected in greatest abundance during periods of relatively warm temperatures. Means of the numbers of *C. imicola*/night/month were plotted against mean minimum and mean maximum monthly temperatures and linear and multiple curvilinear regression analyses were performed. A plot of the geometric mean (ln[no. *C. imicola* per trap night + 1]) versus minimum air temperature at 2° intervals is presented in Fig. 1. The geometric means of *C. imicola* numbers appeared to be linearly correlated with minimum temperature, and the minimum daily temperature at which greatest numbers are collected is at least 18°C (Fig. 1). For maximum daily air temperatures the model fit a 2nd-order polynomial (Fig. 2). The calculated optimal maximum temperature is at least 38°C.

In the province of Cádiz only 3 of 10 traps collected more than one *C. imicola* per trap night. However, this was the only province where adults of this species were captured during every month

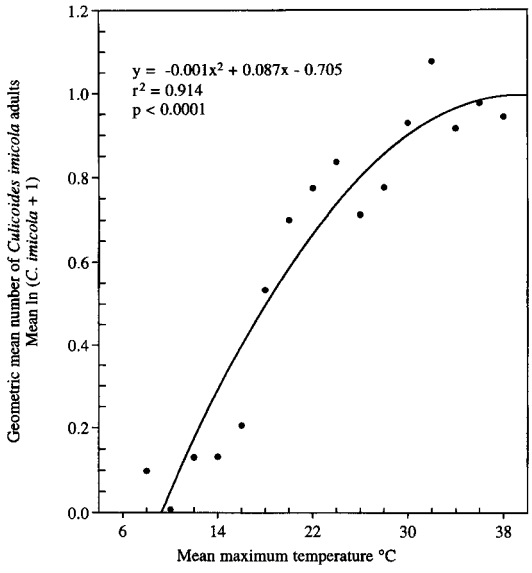

Fig. 2. Geometric mean number of *Culicoides imicola* per trap night versus mean maximum daily temperature.

of the year. Adult *C. imicola* were also found throughout the year in Nigeria (Herniman et al. 1983) and Israel (Braverman et al. 1985). Although the species was collected from November through January in Rhodes, the suggestion was made that this species was probably present in the adult stage throughout the year on both Rhodes and Cyprus (Boorman 1986). In the present study, *C. imicola* was found during only a portion of the year in the province of Huelva, which is the province closest to Algarve and Alentejo in Portugal, where the species was reported throughout the year by Capela et al. (1993).

Culicoides imicola is basically a tropical and subtropical species (Mellor et al. 1989). Although there certainly may be other contributing factors, the presence of a large population of *C. imicola* in western Andalucía and southern areas of the Mediterranean region may be due to the warm temperatures in these areas, whereas the absence of *C. imicola* from other areas of the Mediterranean could be associated with the fact that temperatures in those places are not high enough to support *C. imicola* populations.

Outbreaks of AHS in Andalucía have appeared in the areas where populations of *C. imicola* have been found, including Sotogrande, Marbella, and Sevilla. However, AHS has not occurred in all the places where *C. imicola* has been reported in Andalusia, for example in Jaén (Ortega and Holbrook 1994).

Previous outbreaks of AHS in southern Spain were always from August to November, at a time when we have shown that *C. imicola* populations are at their peak. The absence of AHS transmission at other times of the year could be due to insufficient numbers of *C. imicola* to support an outbreak, incompetence of *C. imicola* to AHS virus, or lack of sufficient host titers of AHS virus.

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