Ecological factors influencing the distributions of *Culicoides imicola* and two species of the Obsoletus Complex in Italy

ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELL'ABRUZZO E DEL MOLISE - TERAMO G. CAPORALE

authors

Annamaria Conte
Armando Giovannini
Maria Goffredo
Carla Ippoliti
Rudy Meiswinkel

Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" Campo Boario 64100 Teramo • ITALY

Introduction

Since 1998 the Mediterranean Basin has experienced the largest outbreak of bluetongue (BT) disease in sheep in the recorded history of man. Some 15 countries have been affected including a number of the Balkan states that have never experienced the disease previously; five serotypes of bluetongue virus (BTV) i.e. BTV-1, -2, -4, -9 and –16 have been implicated. Of particular importance is that many of these outbreaks have penetrated into regions where the principal vector of the disease, the Afro-asiatic *Culicoides imicola* Kieffer, 1913, is absent. This clearly implicates novel vectors in the transmission and spread of bluetongue in Europe and is an issue of particular concern to national veterinary authorities due to its debilitating effects on the trade in livestock.

In 2003 two separate studies in Italy definitively demonstrated the additional involvement of Palaearctic species of *Culicoides* in the transmission of bluetongue when BTV-2 was isolated from five pools of field-collected *C. pulicaris* (Linneaus), 1758 in Sicily, and BTV-2 and BTV-9 from three mixed species pools of *C. obsoletus* (Meigen), 1818 and *C. scoticus* Downes & Kettle, 1952 in south-central peninsular.

Since 2000 an extensive light-trap survey has been operational in Italy; more than 250 Onderstepoort-type blacklight traps are being used to sample *Culicoides* weekly throughout the year and, to date, has resulted in more than 35 000 collections.

We here map the distributions of *C. imicola* and the Obsoletus Complex and attempt also to identify those ecological characteristics that appear to influence their occurrence in nature.

Materials and Methods

Geographical basis

The 8103 municipalities of Italy have been selected as discrete geographic units. All the environmental and statistical analyses performed are based on the administrative boundaries of the municipalities.

Data collection and manipulation

Between the years 2000 to 2004 > 35 000 *Culicoides* light-trap collections have been made across Italy. All have been analysed for the presence/absence and abundance of *C. imicola*; in addition 3 000 of these collections were analysed for the Obsoletus Complex.

All catches were grouped according to their municipality and the maximum abundance of each species recorded. Abundance distributions for the two vector groups were calculated through the Inverse Distance Weighted (IDW) interpolation and only data collected during the warmer months of greatest insect activity (May to November) were considered; 1 473 municipalities have been used in the interpolation of *C. imicola* and 602 for the Obsoletus Complex. Subsequently, two groups of 100 municipalities each, in which the 100 largest collections of *C. imicola* and the 100 largest collections of the Obsoletus Complex had been made, were selected; this choice was based on the assumption that they would reveal the areas in which the vectors were biologically 'successful'.

The independent environmental and climatic variables included in the statistical analysis were: mean minimum temperature between May and November for the years 2000 – 2004; mean altitude above sea level; average terrain slope; percentage area of a given municipality covered by forests (broad leaved, coniferous and mixed forest); average of the Normalised Difference Vegetation Index (NDVI - this being a specific measure of chlorophyll abundance and light absorption) in the period May to November (years 2000 – 2002) and the Aridity Index (rainfall/potential evapotranspiration Thornthwaite – PET). Climatic data were obtained from the Italian Air Force Meteorological Service accumulated between 2000 and 2004 from 105 weather stations distributed almost equidistantly across Italy. These data were interpolated to estimate the average value for each municipality through geostatistical interpolation by ordinary kriging using the Geostatistical Analyst module of ESRI ArcGis ™. The average values were then extracted for each municipality by the Spatial Analyst module of ESRI ArcGis ™.

Statistical analyses

The non-parametric Mann-Whitney test was applied to the independent variables listed above to verify whether a statistically significant difference existed between the municipalities with the 100 largest catches of *C. imicola* (group 1) and the Obsoletus Complex (group 2) respectively. To identify the variables most important for distinguishing municipalities with the highest catches of the two vectors a Discriminant Analysis was performed. Due to high correlation amongst variables, the Discriminant Analysis was applied to the independent factors, these identified through Factor Analysis. Principal component methods were used to identify the common factors, while the varimax method was used for orthogonal rotation.

Figure 1a. Elevation above sea level

Figure 1b. Aridity Index

Figure 1c. Mean minimum temperature (May- November)

Figure 1d. Normalised Difference Vegetation Index (May – November)

Results

Figure 1a is a general topographic map of Italy combining the two considered variables of altitude and slope; **Figure 1b** maps the aridity index; **Figure 1c** shows the mean minimum temperature and **Figure 1d** maps the 200 average NDVI. In **Figures 2** and **3** the geographical distributions of the two *Culicoides* vector groups are shown. In **Figure 4** the geographical distribution of the municipalities with the 100 largest catches of *C. imicola* (in red), and of the Obsoletus Complex (in blue), is shown. These are superimposed on the distribution of broad leaved, coniferous and mixed forests (in green). The Mann-Whitney test was statistically significant ($p<0.01$) for all the variables meaning that a real difference existed between the values calculated for the two insect vector groups (**Table 1**). Using Factor Analysis six variables were processed and led to the identification of two principal factors, referred to broadly as 'abiotic' and 'biotic' (**Table 2**); these explained 84% of the total variability. The 200 observations were then plotted against the first ('abiotic') and second ('biotic') factors (**Figure 5**). The Discriminant Analysis performed on the two factor scores calculated for each observation gave the standardized discriminant function coefficients as shown in **Table 3**. Since the two factors are independent, the standardized coefficients are a reliable index of the importance of each factor. The total percentage of municipalities correctly classified as to its 'dominant' insect vector species was 87.5% (**Table 4**).

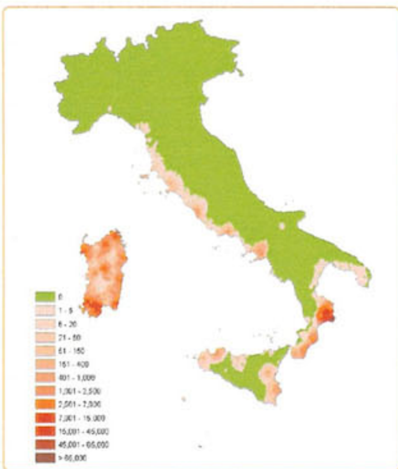


Figure 2. The geographic distribution of *Culicoides imicola* in Italy divided into 13 abundance classes (2000 – 2004)

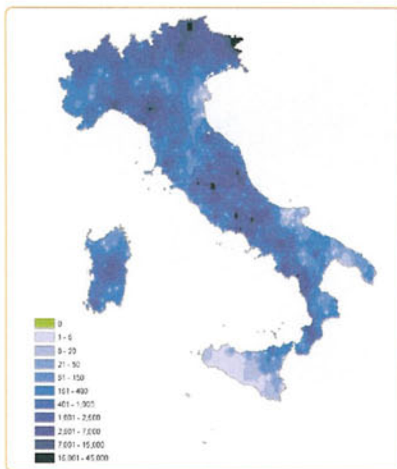


Figure 3. The geographic distribution of the Culicoides obsoletus species complex in Italy divided into 11 abundance classes (2000 – 2004)

Figure 4. Geographic distribution of the 200 largest catches of *Culicoides imicola* and of the Obsoletus Complex collected between the years 2000 and 2004; green areas depict forested regions

Table 1. Mean values and 95% confidence intervals of the six variables for the two insect vector groups for 200 municipalities in Italy

Variable	Mean values and 95% confidence intervals *C. imicola*	Obsoletus Complex
Altitude	207.5 (176.8 – 238.2)	587.1 (488.3 – 685.9)
Percentage of forest	11.2% (8.9% - 13.7%)	31.6% (27.6% – 35.5%)
Terrain slope	5.3 (4.7 – 5.9)	9.6 (8.5 – 10.5)
Minimum temperature	16.9 (16.8 – 17.0)	14.2 (13.9 – 14.6)
NDVI	0.29 (0.27 – 0.31)	0.47 (0.45 – 0.49)
Aridity index	0.87 (0.74 – 1)	23.6 (14.32 – 32.87)

Table 2. Rotated factor matrix – varimax method

	Factor 1	Factor 2
Min. temperature	-0.62	-0.57
Aridity index	0.96	0.10
Slope	0.78	0.45
Altitude	0.91	0.32
NDVI	0.25	0.89
Forest %	0.21	0.88

Table 3. Standardized coefficients of Discriminant Analysis

Factor 1	Coefficient
'abiotic'	0.546
'biotic'	0.993

Table 4. Classification of observed and predicted insect vector groups

Observed group	Predicted group *C. imicola*	Obsoletus Complex	*Total*
C. imicola	91	9	100
Obsoletus Complex	16	84	100
Total	107	93	200

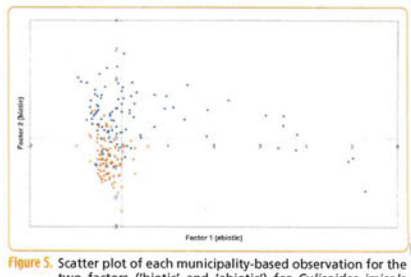


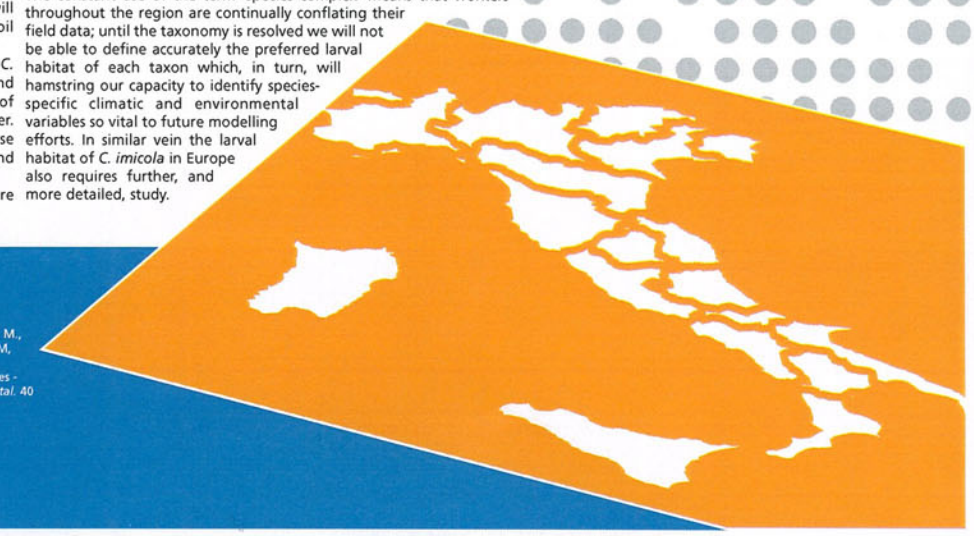
Figure 5. Scatter plot of each municipality-based observation for the two factors ('biotic' and 'abiotic') for *Culicoides imicola* (red) and for the Obsoletus Complex (blue).

Discussion

With an insect-borne disease it is critical to understand the ecology of the vectors involved if one wishes to model accurately their distribution in both time and space. Ultimately, the availability of the appropriate breeding substrate is key to the production of viable adult *Culicoides* populations in the field. By definition each species occupies a specific niche in nature meaning that each will differ from the next in one or more aspects of its life cycle. These differences have to be identified and quantified. Currently the trend is to develop predictive risk maps but most have shortcomings because the ecological constraints operative upon a particular vector are poorly known. It is also true that countries facing the threat of bluetongue require actual, and not predictive, data. Thus the Italian Ministry of Health opted for a detailed field survey to obtain real data on vector distributions and prevalences, and to begin the identification of the multifactorial environmental influences operational on each. Broadly the results show that:

- The distribution of *Culicoides imicola* in Italy is restricted and fragmented due to the interaction of many factors including mean minimum temperature, altitude, terrain slope, aridity and soil type [1]. Ultimately, this unusual distribution of *C. imicola* in Italy is not deemed to reflect a recent, and still incomplete, invasion as has been mooted recently [2];
- Of the 19 225 foci of BT reported in Italy over the last five seasons, 97% occurred on the island of Sardinia where *C. imicola* is found in great abundance. Furthermore, because its distribution elsewhere in Italy also matched closely that of the disease, *C. imicola* is still considered the principal vector of BT;
- To date the northernmost record for *C. imicola* in Italy is Piacenza at 44°46'N; because only single specimens are captured at these high latitudes each season we tend to the view that *C. imicola* is not spreading rapidly northwards into Europe. However, under an ameliorating climate, it could advance northwards but this will likely happen slowly and only into areas where the appropriate soil conditions prevail;
- The Obsoletus Complex (represented by *C. obsoletus* and *C. scoticus*) occurs widely and abundantly, both latitudinally and altitudinally, in Italy [3]. Its occurrence correlates with that of forests so fitting previous observations that it breeds in forest litter. Its distribution deviated substantially from that modelled by Purse *et al.* [4] based upon data assembled in Sicily but where we found the Obsoletus Complex to be depauperate;
- When the 100 largest collections of the two vector groups were mapped the respective distributions proved strongly disjunct. In the main it can be said that *C. imicola* favours open habitats (exposed to sunlight) whereas the Obsoletus Complex is restricted to those that are sheltered (or shaded). One possible effect is that fewer generations of the latter will be produced in a season so lowering its potential to transmit an orbiviral disease to livestock.

The unresolved taxonomy of the Obsoletus and of the Pulicaris Complex in Europe, which together comprise at least 18 species [5], is hindering the development of reliable taxon-specific distribution maps. The constant use of the term 'species complex' means that workers throughout the region are continually conflating their field data; until the taxonomy is resolved we will not be able to define accurately the preferred larval habitat of each taxon which, in turn, will hamstring our capacity to identify species-specific climatic and environmental variables so vital to future modelling efforts. In similar vein the larval habitat of *C. imicola* in Europe also requires further, and more detailed, study.



References

1. Conte A., Ippoliti C., Calistri P., Pelini S., Savini L., Salini R., Goffredo M., Meiswinkel R. (2004). Towards the identification of potential infectious sites for bluetongue in Italy: a spatial analysis approach based on the distribution of *C. imicola*. *Vet. Ital.* 40 (3): 311-315.
2. Purse B. V., Mellor P. S., Rogers D. J., Samuel A. R., Mertens P. P. C. and Baylis M. (2005). Climate change and the recent emergence of bluetongue in Europe. *Nature Reviews, Microbiology* 3: 171-181.
3. Gomulski LM, Meiswinkel R, Delécolle J.-C., Goffredo M, Gasperi G (2005) Phylogenetic relationships of the subgenus *Avaritia* Fox, 1955 including *Culicoides obsoletus* (Diptera, Ceratopogonidae) in Italy based on internal transcribed spacer 2 ribosomal DNA sequences. *Systematic Entomology*: Online: DOI: 10.1111/j.1365-3113.2005.00286.x.
4. Purse B. V., Tatem J., Caracappa S., Rogers D. J., Mellor P. S., Baylis M. and Torina A. (2004). Modelling the distributions of *Culicoides* bluetongue virus vectors in Sicily in relation to satellite-derived climate variables. *Medical and Veterinary Entomology* 18: 90-101.
5. Meiswinkel R, Gomulski L. M., Delécolle J.-C., Goffredo M, Gasperi G . Taxonomy of *Culicoides* vector complexes - unfinished business. *Vet. Ital.* 40 (3): 151 – 159.

Graphic design: Sandro Santarelli • IZSA&M • 2005