Novel environmental factors influencing the distribution and abundance of *Culicoides imicola* and the Obsoletus Complex in Italy

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Summary

Bluetongue (BT) is an orbiviral disease of ruminant livestock. In Europe, the insect vectors implicated in BT virus transmission are *Culicoides imicola*, *C. obsoletus*, *C. scoticus*, *C. pulicaris* and *C. dewulfi*. *C. imicola* – an Afro-Asiatic species – is believed to account for approximately 90% of BT virus transmission; the latter four species are endemic to the Palaearctic region and are becoming increasingly important as the zone of BT virus infection in Europe moves northwards. As *Culicoides* distribution and ecology is critical in the spread of BT, it is essential to map their geographic ranges and to determine the ecological and climatic factors influencing their occurrence. Since 2000, more than 50 000 light trap collections have been made across Italy and the presence/absence and abundance of *C. imicola* ascertained; 3 000 of these collections were also screened for two species (*C. obsoletus*, *C. scoticus*) of the Obsoletus Complex. To gain further insights into vector breeding habitats, spatial analysis techniques and statistical methodologies were used to investigate the association between the trap collection (municipality [polygons] and geographic point level) data and environmental and climatic variables. For the first dataset, 100 municipalities with the largest collections of *C. imicola* and 100 of the Obsoletus Complex, were selected and the average values of six independent climatic/environmental variables were calculated for each polygon. Factor analysis identified two principal factors explaining 84% of the total variability in climatic and environmental variables; 87.5% of trap collections were correctly classified by a discriminant analysis model. *C. imicola* was found to predominate in more sparsely vegetated habitats. Obsoletus Complex species were associated with a more densely vegetated habitat. For the second dataset, the presence/absence of *C. imicola* at 172 sites in the region of Calabria (southern Italy) was investigated using 13 climatic, environmental and pedological variables. Factor analysis identified four factors; 89.5% of trap collections were correctly classified by a discriminant analysis model. Proximity to water-holding, clay soils was the most important factor explaining the markedly disjoint distribution of *C. imicola* in the 'toe' of Italy. Soil type may explain the patchy distribution of *C. imicola* elsewhere in Mediterranean Europe.

Keywords

Bluetongue, Clay, *Culicoides imicola*, Forest, Habitat, Italy, Obsoletus Complex, Shade, Soil, Sunlight, Vector.

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Effetti di nuovi fattori climatici ed ambientali sulla distribuzione di *Culicoides imicola* ed Obsoletus Complex in Italia

Riassunto

La bluetongue (BT), è una malattia infettiva dei ruminanti trasmessa da insetti vettori. In Europa gli insetti vettori implicati nella trasmissione della malattia sono Culicoides imicola, C. obsoletus, C. scoticus, C. pulicaris *e* C. dewulfi. C. imicola *– una specie Afro-Asiatica – è responsabile per il 90% della trasmissione del virus della BT; le restanti quattro specie, endemiche nella regione Paleartica, stanno tuttavia aumentando di importanza con l'avanzamento verso Nord del virus della BT. La distribuzione e l'ecologia dei* Culicoides, *con i fattori climatici ed ambientali che influenzano la loro presenza, risultano cruciali per comprendere la diffusione della BT. In Italia, dal 2000 ad oggi, sono state effettuate più di 50.000 catture analizzate per presenza/abbondanza di* C. imicola; *per 3.000 catture è stata inoltre definita la presenza ed abbondanza di Obsoletus Complex (*C. obsoletus, C. scoticus*). Al fine di identificare l'associazione esistente tra variabili climatiche/ambientali e la presenza dei due vettori, sono stati analizzati due set di dati: il primo a livello poligonale (comuni italiani), il secondo a livello puntuale (aziende). Per il primo dataset sono stati individuati rispettivamente i 100 comuni con più abbondanti catture di* C. imicola *e 100 comuni con più abbondanti catture di Obsoletus Complex. Per ciascun comune sono stati calcolati i valori medi di 6 variabili climatiche/ambientali. Per verificare quali variabili fossero più significative nella discriminazione dei due gruppi è stato utilizzato un modello di analisi discriminante, applicato ai due fattori individuati mediante analisi fattoriale (per eliminare la forte correlazione esistente tra le variabili); l'87,5% dei 200 comuni è stato classificato correttamente dal modello discriminante.* C. imicola *è risultata predominante in aree con scarsa vegetazione, mentre l'Obsoletus Complex è risultato associato con habitat in cui la vegetazione è particolarmente intensa. Nel secondo dataset sono stati analizzati 172 siti di cattura (classificati come positivi o negativi per* C. imicola*) nella regione Calabria utilizzando 13 variabili climatiche, ambientali e podologiche. L'analisi fattoriale ha ridotto a quattro fattori principali le 13 variabili correlate e l'analisi discriminante applicata ai quattro fattori ha classificato correttamente l'89,5 % dei siti di cattura. Il fattore più importante nella classificazione di siti positivi e negativi per* C. imicola *è risultato la vicinanza a suoli di natura argillosa, capaci di trattenere più acqua rispetto ai suoli di natura sabbiosa.*

Parole chiave

Argilla, Bluetongue, *Culicoides imicola*, Esposizione, Foreste, Habitat, Italia, Obsoletus Complex, Ombra, Suoli, Vettori.

Introduction

Between 1998 and 2006, the Mediterranean Basin has been at the centre of the largest outbreak of bluetongue (BT) disease of sheep ever recorded. About 15 countries surrounding the Basin have been affected, including a number of Balkan states that had not experienced the disease previously. Outbreaks have involved 5 of the 24 known serotypes of bluetongue virus (BTV), BTV-1, -2, -4, -9 and -16, in south-eastern Europe. BTV-9 has advanced northwards and eastwards into areas where the main vector of BT, *Culicoides imicola*, does not occur.

The occurrence of BT in regions where *C. imicola* is absent has implicated novel *Culicoides* vectors in the transmission of the disease, and BTV has been isolated from species of the Obsoletus (11, 26, 27) and the Pulicaris (8) Complexes. In August 2006, BTV -8 was unexpectedly detected in north-western Europe (The Netherlands, Germany, Belgium, France and Luxemburg) (29). *C. imicola* has never been reported in this area of northern Europe. Endemic *Culicoides* are likely playing an increasingly important role in the transmission of BT disease in the Palaearctic region.

Since *Culicoides* vectors are vital in the dissemination of BT, it is essential to accurately map their geographic ranges. Species ranges are largely dictated by climatic and environmental factors, many of which have been identified (especially for *C. imicola*) and used to develop predictive vector distribution maps (3, 6, 9, 10, 25, 28, 30). However, these maps have limited accuracy compared to 'ground-truthing'. Lack of accuracy might be due to failure to account for the strong influence of optimal breeding habitat availability. The breeding habitats of the five suspect or confirmed vectors of BTV in Europe appear to differ fundamentally. Variables related to soil associated with these habitats therefore should be considered in predictive modelling of species range. In this study, some novel environmental factors – such as soil texture – are examined and discussed.

Materials and methods

To identify the environmental and climatic variables affecting the distribution of *C. imicola* and the Obsoletus Complex in Italy, two studies were conducted. First, the distributions of *C. imicola* and of the Obsoletus Complex were compared using long-term light trap data obtained at municipality level (polygons), and second, using geographic point data (coordinates of the farms where traps were placed), sites in the region of Calabria found to be either positive or negative for the presence of *C. imicola,* were compared.

Culicoides imicola and the Obsoletus Complex

Entomological data collection

Between 2000 and 2004, more than 38 000 light-trap collections were made in Italy according to standardised surveillance procedures (14). All collections were analysed for the presence/absence and abundance of *C. imicola*. Approximately 3 000 of these collections were also analysed for the Obsoletus species Complex. Only collections made during the warmer months of greatest insect activity (May to November) were considered. Data collected for *C. imicola* in 1 473 municipalities and for the Obsoletus Complex in 602 municipalities were used to select two groups, namely:

- 100 municipalities with the 100 largest collections of *C. imicola*
- 100 municipalities with the largest collections of the Obsoletus Complex.

The two groups were chosen on the assumption they would reveal areas in which the vector taxa were 'biologically successful'. When a municipality was represented by both vector taxa, only the dominant taxon was selected.

Climatic and environmental data

The association between vectors trapped in the two groups of municipalities and the following variables were analysed:

- mean minimum temperature between May and November for the years 2000-2004
- mean altitude above sea level
- average terrain slope (degrees)
- percentage area covered by forests (broad-leaved, coniferous and mixed forests) (Corine Land Cover version 12/2000 Environmental European Agency) (www.eea.eu.int)
- average of the normalized difference vegetation index (NDVI) – this being a specific measure of chlorophyll abundance and light adsorption – in the period between May and November (2000-2002) (Royal Netherlands Meteorological Institute [KNMI])
- aridity index (rainfall/potential evapotranspiration Thornthwaite – PET) (Environmental European Agency) (www.eea.eu.int)

Climate data were obtained from the Italian Air Force Meteorological Service from 105 weather stations distributed almost equidistantly across Italy. These data were geostatistically interpolated through ordinary kriging (Geostatistical Analyst module of Environmental Systems Research Institute [ESRI] ArcGis™) and the average values for each municipality were obtained. The remaining variables – available in grid format – were resampled at the same 250 m × 250 m resolution and analysed through ArcGis™ Spatial Analyst; the average values for each municipality were then extracted.

The autocorrelation of trap catches was not explicitly modelled. Due to the shape of the Italian territory, and the presence of some major islands (Sicily and Sardinia) and topography, such as the Apennines which divide longitudinally the entire Italian mainland, developing an autocorrelation model for the entire country is problematic.

Statistical analyses

To identify the variables that may possibly explain the differences between the respective vector habitats of *C. imicola* and the Obsoletus Complex, a discriminant analysis was performed. A Spearman correlation matrix was also calculated for these variables. Due to high correlation amongst variables, independent factors identified through factor analysis – a statistical technique used to determine the degree of relationship amongst interrelated variables (15) – were analysed. The principal component method was used to identify the common factors, while the varimax method was used for orthogonal rotation. All analyses were performed in SPSS®11.0.

Comparison of *Culicoides imicola* positive and negative sites in the region of Calabria

Entomological data collection

Between 2000 and 2006, more than 50 000 light-trap collections were made in Italy. Only collections made in the region of Calabria during the warmer months (May to November) were considered. For 172 geo-referenced catch sites, multiple catches were performed and the maximum value of *C. imicola* was taken into consideration to classify the site as positive or negative.

Climatic, environmental and pedological data

Associations between positive and negative sites for *C. imicola* in the Calabria region and the following variables were analysed:

- mean minimum temperature between May and November for the years 2000-2004
- elevation (metres) above sea level
- terrain slope (degrees)
- average of the NDVI – in the period May to November (2000-2002) (KNMI)
- aridity index (rainfall/PET) (Environmental European Agency) (www.eea.eu.int).
- distance of each site from forests
- slope direction (exposure to sun)
- percentage of soil organic material content (*Agenzia Regionale per lo Sviluppo e per i Servizi in Agricoltura ARSSA – Regione Calabria*)
- soil water content (volume of water per unit volume of soil) (*Agenzia Regionale per lo Sviluppo e per i Servizi in Agricoltura ARSSA – Regione Calabria*)
- percentage of clay, silt, sand (soil texture) (*Agenzia Regionale per lo Sviluppo e per i Servizi in Agricoltura ARSSA – Regione Calabria*)
- distance from fine texture soils.

Statistical analyses

For the statistical analyses, the 172 sites were classified into two groups: one group of 102 *C. imicola*-negative sites and one of 70 *C. imicola*-positive sites. To identify the variables distinguishing between positive and negative sites, a factor analysis was used to remove multicollinearity amongst the independent variables, followed by a discriminant analysis. The principal component method was used to identify the common factors, while the varimax method was used for orthogonal rotation. All analyses were performed in SPSS®11.0.

Results

Culicoides imicola and the Obsoletus Complex

In Figure 1, the 100 largest catches of *C. imicola* (in red) and the 100 largest catches of the Obsoletus Complex (in blue) are mapped and superimposed on the distribution of broad-leaved, coniferous and mixed forests (in green). The correlation matrix amongst the six climatic and environmental variables is shown in Table I.

Factor analysis identified two principal factors: 'abiotic' and 'biotic' (Table II). The former includes minimum temperature, the aridity index, terrain slope and altitude; the latter percentage of forest and NDVI. Together, these two factors explained 84% of the total variability. To show how the observations are scattered, factor scores were calculated by multiplying standardised values and factor coefficients and plotted (Fig. 2).

A discriminant analysis on the two factors (normally distributed according to the chi-square goodness of fit test) gave the standardised coefficients shown in Table III.

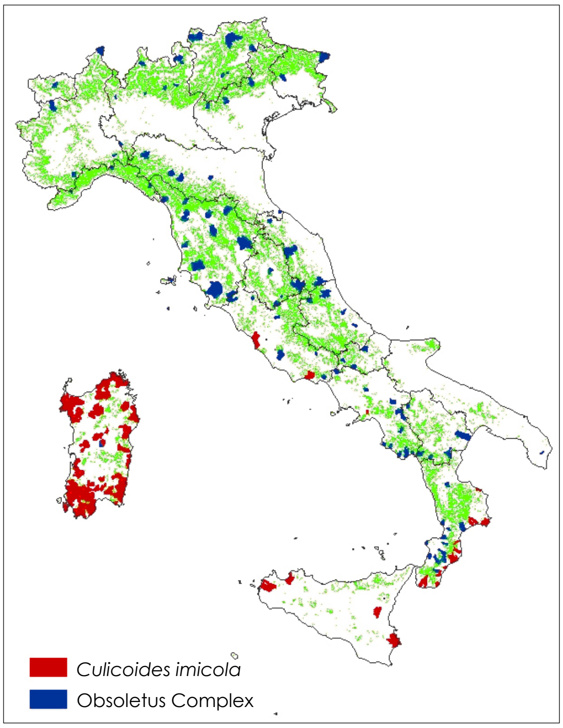

Figure 1
Distribution of the 100 largest catches of *Culicoides imicola* and of the Obsoletus Complex collected in Italy, 2000-2004
Green represents the combined distribution of broad-leaved, coniferous and mixed forests

Since the two factors are independent, they offer a reliable index of the importance of each factor. Overall, the 'dominant' insect vector species in 87.5% of the municipalities were correctly classified by the discriminant analysis function (Table IV).

Table II
Rotated factor matrix – varimax method for six independent variables at the sites of the 100 largest catches of *Culicoides imicola* and of the Obsoletus Complex in Italy, 2000-2004

Variable	Factor 1	Factor 2
Min. temperature	–0.62	–0.57
Aridity index	0.96	0.10
Slope	0.78	0.45
Elevation	0.91	0.32
NDVI	0.25	0.89
Forest (%)	0.21	0.88

NDVI normalized difference vegetation index

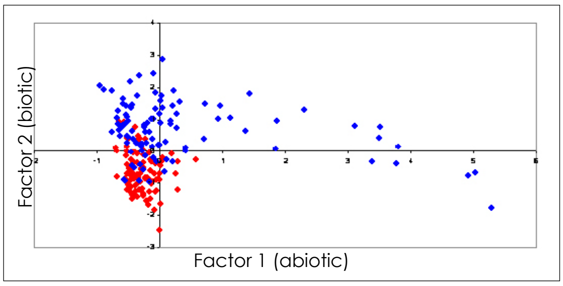

Figure 2
Scatter plot of each municipality-based observation for the two factors ('biotic' and 'abiotic') for *Culicoides imicola* (red) and for the Obsoletus Complex (blue)

Table III
Standardised coefficients of a discriminant analysis model of environmental variables (identified by factor analysis; see Table II) of the sites of the 100 largest catches of *Culicoides imicola* and of the Obsoletus Complex in Italy, 2000-2004

Factor	Coefficient
Factor 1	0.546
Factor 2	0.993

Table I
Spearman's correlation matrix of six independent variables at the sites of the 100 largest catches of *Culicoides imicola* and of the Obsoletus Complex in Italy, 2000-2004

Variable	Min. temperature	Aridity index	Slope	Altitude	NDVI	Forest (%)
Min. temperature	–	–0.81*	–0.43*	–0.53*	–0.71*	–0.51*
Aridity index		–	0.70*	0.78*	0.82*	0.61*
Slope			–	0.80*	0.65*	0.65*
Altitude				–	0.77*	0.62*
NDVI					–	0.73*
Forest (%)						–

NDVI normalized difference vegetation index
* two-tailed $p < 0.01$

Table IV
Classification of observed catches of *Culicoides imicola* and of Obsoletus Complex in Italy, 2000-2004, and predicted catches using a discriminant analysis model based on environmental factors

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

Comparison between *Culicoides imicola* positive and negative sites in the region of Calabria

The distribution of positive and negative *C. imicola* sites in the region of Calabria is shown in Figure 3a. A factor analysis of 13 variables identified four principal factors (Table V): the first factor included elevation, NDVI, minimum temperature, slope, aridity index, distance from forest and percentage of soil organic material; the second factor included percentage of clay, silt, sand and soil water content; the third factor was distance from fine texture soils, and the last factor was slope direction (exposure to sun). The discriminant analysis performed using these four independent factors (three factors were normally distributed according to the chi-square goodness of fit test, only for one [exposure to sun] the hypothesis of normality was rejected) gave the standardised coefficients shown in Table VI; the *C. imicola* status of 89.5% of the sites were correctly classified (Table VII).

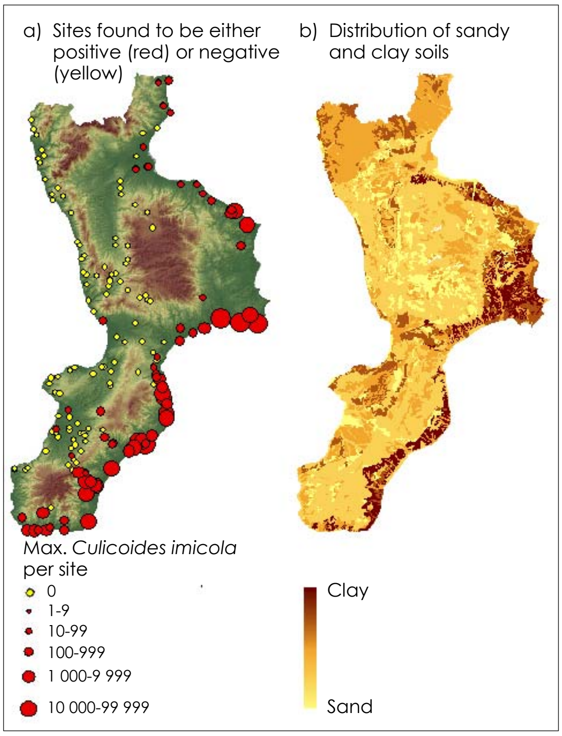

Figure 3
Distribution of *Culicoides imicola* and of sandy and clay soils in the Calabria region, southern Italy

Table V
Rotated factor matrix – varimax method for 13 independent variables at the positive and negative sites for *Culicoides imicola* in the Calabria region of Italy, 2000-2006

Variable	Factor 1	Factor 2	Factor 3	Factor 4
Elevation	0.88	–0.12	0.01	–0.09
Organic material	0.80	–0.07	0.05	–0.15
NDVI	0.75	–0.25	0.20	–0.12
Distance from forests	–0.67	0.02	–0.12	–0.15
Aridity index	0.65	–0.11	0.44	0.07
Min. temperature	–0.53	0.30	–0.48	–0.12
Slope	0.43	–0.14	–0.02	0.34
Sand	0.10	–0.97	0.11	–0.01
Silt	–0.06	0.89	–0.12	–0.06
Clay	–0.17	0.84	–0.09	0.08
Water content	–0.17	0.74	–0.01	–0.18
Distance from moisture soil	0.04	–0.01	0.92	–0.07
Slope direction	–0.10	–0.04	–0.01	0.91

NDVI normalized difference vegetation index

Table VI
Standardised coefficients of a discriminant analysis model of environmental variables (identified by factor analysis; see Table V) of the positive and negative sites for *Culicoides imicola* in the Calabria region of Italy, 2000-2006

Factor	Coefficient
Factor 1	0.784
Factor 2	–0.348
Factor 3	0.902
Factor 4	–0.436

Table VII
Classification of observed and predicted positive and negative catches of *Culicoides imicola* in the Calabria region of Italy, 2000-2006, using a discriminant analysis model based on environmental factors

Observed group \ Predicted group	*Culicoides imicola* negative	*Culicoides imicola* positive	Total
Culicoides imicola negative	92	10	102
Culicoides imicola positive	8	62	70
Total	100	72	172

Discussion

Culicoides imicola and the Obsoletus Complex

Comparative mapping of the 100 largest collections of *C. imicola* and of the Obsoletus Complex showed that they were strongly disjoint, facilitating the identification of some biotic and abiotic factors that promote (or suppress) the proliferation of these two vectors in the field. *C. imicola* was mostly found to be constrained within these factors, indicating that its optimal breeding conditions are not widely available in Italy. Its presence in areas of low NDVI (less vegetated habitats) indicates that this species favours more open areas that are exposed to full sunlight. This corresponds to what is known about its breeding habitat both in Africa (19, 22) and in the Mediterranean region (5, 20).

The broader distribution of the Obsoletus Complex – especially across abiotic factors – demonstrates its tolerance of a wider range in temperature, altitude and terrain slope. Unlike *C. imicola*, the Obsoletus Complex is present in much of Italy and can be found in both flat and steeply sloping terrain and at altitudes in excess of 2 000 m. Furthermore, the predominance of the Obsoletus Complex in an elevated NDVI range (average NDVI 0.47 [lower confidence limit: 0.45; upper confidence limit: 0.49] versus an average NDVI of 0.29 [lower confidence limit: 0.27; upper confidence limit: 0.31] for *C. imicola*) indicates that it favours breeding habitats that are more shielded from direct solar radiation. Indeed, its distribution fits closely that of deciduous broadleaved and mixed broadleaved/ coniferous forest and supports previous observations that it, and closely related species, breed preferentially in forest leaf litter (1, 13, 16).

It is important to stress that in the municipality-based datasets, open and closed habitats can co-occur. This explains why *C. imicola* and the Obsoletus Complex will often be found sympatrically, but very seldom co-dominantly, in any given locality; this is true also in mainland Spain (23), in Morocco (4), in Portugal (7), on the Balearic islands (21) and on Corsica (2). This disjoint distribution across the Mediterranean Basin argues against the proposal that *C. imicola* and the Obsoletus Complex share a '…common habitat' (24).

Comparison between *Culicoides imicola* positive and negative sites in the region of Calabria

In previous studies, temperature, elevation, humidity, rainfall and NDVI were amongst a number of variables used to predict the prevalence and the presence of *C. imicola* across the Mediterranean region (3, 6, 9, 10, 25, 28, 30). The accuracy of these predictions could be increased by also evaluating other environmental variables. Novel variables can be identified by analysing situations in which the distribution of *C. imicola* is interrupted profoundly over short spatial distances, thereby reducing the influence of climate. An example is the region of Calabria, where *C. imicola* occurs abundantly in the east but is almost entirely absent in the west. In addition, this disjoint distribution has remained stable since it was first discovered in 2001.

In areas of South Africa, *C. imicola* has been observed to be absent from sandy coastal areas despite a mild prevailing climate (including adequate rainfall) and a large resident animal biomass (17). The absence of this species has been linked causally to soils with a sandy texture, which are known to have depleted moisture levels (especially in the surface layer) and to lack vital nutrients. Moisture is critical to the survival of *C. imicola* larvae, whilst nutrients are essential for their development and for the completion of their 7-10 day life-cycle. Soil texture refers to the relative proportions of sand-, silt- and clay-sized inorganic particles comprising a sample of soil and may be grouped broadly into three sizes: clay (<0.002 mm), silt (0.02 to 0.002 mm) and sand (2.0 to 0.02 mm) (12). The water-holding capacity of finely textured clay soils is greater than that of coarsely textured sandy soils (that have large, well-connected pore spaces which promote greater moisture permeability). In the current study, distance from moisture-retentive soils was the most important factor for distinguishing between positive and negative *C. imicola* sites in the region of Calabria. As illustrated in Figure 3, moisture-retentive clay soils occur almost exclusive along the eastern coastline of Calabria (Fig. 3b). This coincides with the distribution of *C. imicola* (Fig. 3a). The high abundances of *C. imicola* mirrors the situation in southern Africa where *C. imicola* becomes highly abundant in areas with clay soils, especially following periods of continuous rain (18). This recurrent pattern of distribution in clay soils at opposite ends of the vast geographic range of *C. imicola* would seem to add weight to the hypothesis that its immature stages thrive best in nutrient-rich, water-holding soils. It could help explain the fragmented distribution of *C. imicola* in both Italy and in other parts of southern Europe that lie below the 45th parallel. If so, it would seem to nullify the argument that the eastern occurrence of *C. imicola* in the 'toe' of Italy is due to its recent – and thus incomplete – invasion of Europe from the south (Africa). However, it is still not known where *C. imicola* breeds 'naturally' in the Mediterranean region. Especially during mid-summer, microclimates foci, such as the banks of drying streams and rivers, or areas of mud associated with animal drinking troughs on farms, may be the key to the persistence of this species.

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