

Coccinellids (Coleoptera: Coccinellidae) in citrus groves in Portugal: listing and analysis of geographical distribution

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Coccinellids were sampled in 88 citrus groves from the centre/south of continental Portugal. Analysis of distribution were performed by the usual indices but also with the help of a new method - SADIE.

38 species were identified, 36 of which are predators. Five coccinellids seem to have aggregated distributions; UTM maps of those species distributions are presented. Among these five, *Nephus reunioni* Fürsch and *Nephus includens* (Kirsch) are the more abundant. Previous studies about these two coccinellids distributions are confirmed: *N. reunioni* is limited to a region of about 80 Km diameter around Lisbon while *N. includens* appears only in Algarve.

Coccidophagous species and a group of *Scymnus* sp. and *Nephus* sp., with unknown food preferences in citrus, are the most important in terms of richness and abundance.

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INTRODUCTION

The culture of Citrus has a long tradition in Portugal and is still one of the most important crops in the country. In 1994 the orchards of various species of citrus covered 24.638 ha, mainly in the south of Portugal (Instituto Nacional Estadística, 1995).

Many phytophagous insects are reported to feed on citrus but only ten are considered as key pests in continental Portugal (CARVALHO, *et al.* in press). Among these we find four Homoptera: *Aleurothrixus floccosus* (MASKELL) (Aleyrodidae), *Planococcus citri* (RISSEO) (Pseudococcidae), *Saissetia oleae* (OLIVIER) (Coccidae) and *Lepidosaphes beckii* (NEWMAN) (Diaspididae).

The development of the crop is subsidised by the European Administration and great

attention is paid to the implementation of Integrated Pest Management (IPM). In fact, the citrus have a complex phytosanitary situation that can be solved with this kind of approach putting together some of the already existing biological control measures and the chemical treatments still needed against some pests.

Among the research projects in progress, biological control of coccids by coccinellids, which are claimed to be the most rich and abundant group of predatory insects in citrus (LONGO & BENFATTO, 1987), is an important topic. The interest raised by this family is also supported by the fact that the first successful and often repeated case of biological control featured a ladybird beetle, *Rodolia cardinalis* (MULSANT) released against *Icerya purchasi* Maskell, a coccid, in 1888. Since

then, other species of ladybird beetles succeeded in controlling several species of coccids around the world (HODEK, 1970, COPPEL & MERTINS, 1977, IPERTI, 1987, MAJERUS, 1994, DIXON, *et al.*, 1997).

The fauna of the coccinellids is well described in Portugal (see RAIMUNDO & ALVES, 1986) but their basic ecology is barely known. However, the mechanisms driving the variations in abundance of predatory species taken in isolation or in a community context are essential to define rational programmes of biological control.

The present work aimed to improve our knowledge on the distribution of the coccine-

lids present in citrus groves from the Centre/South areas of continental Portugal. The ladybirds present in citrus groves from the most important regions of production were sampled and their geographical distribution established.

In order to accomplish these objectives we used some faunistic methodologies seldom employed for this kind of applied research. UTM (Universal Transverse Mercator) grid maps and analysis of distribution patterns are among them. To the traditional measures of animal aggregation (Dispersion Indices) we added a new methodology –SADIE (Spatial Analysis by Distance Indices)– which allo-

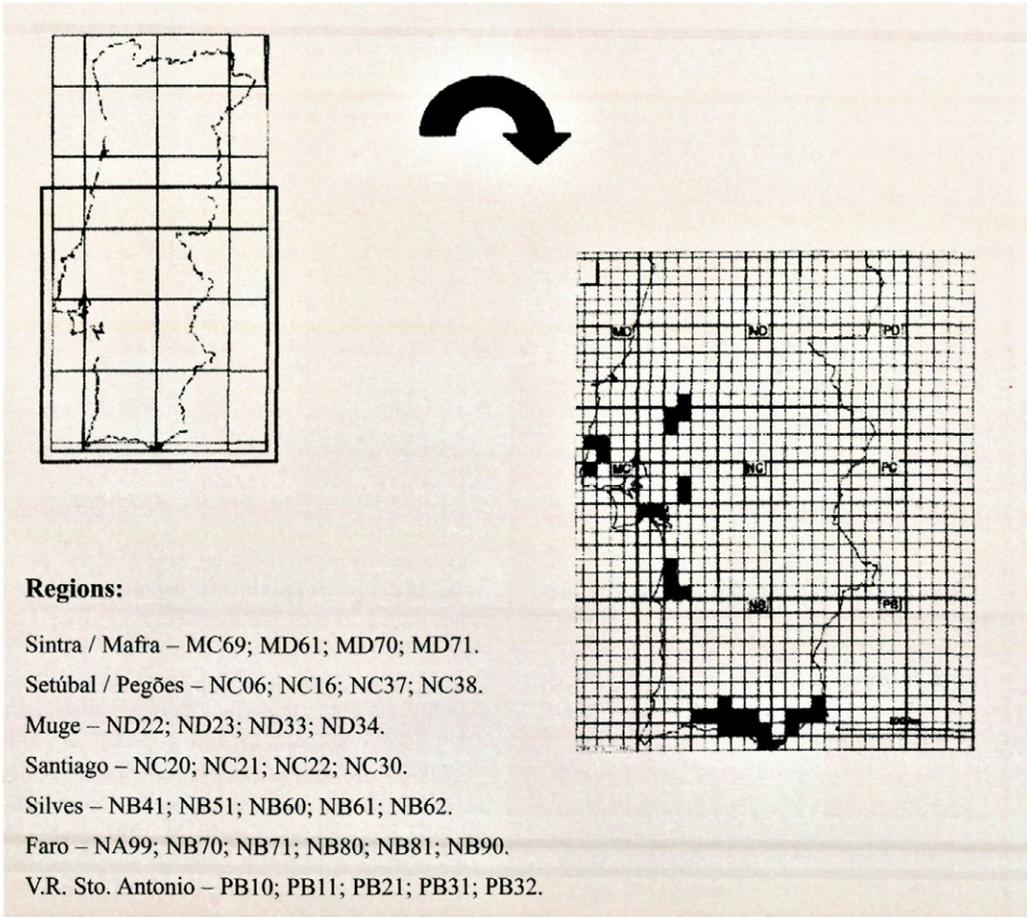


Fig. 1. - Sampling of coccinellids in 88 citrus groves in Portugal. Groves are located in 32 UTM squares of 10 km., corresponding to 7 main regions.

wed us to take into account the available spatial information in the sample (PERRY, 1995 *a, b*).

MATERIAL AND METHODS

Samples of adult coccinellids were collected from 1991 to 1994 in 88 citrus groves located in 32 UTM squares of 10 Km in Portugal (Fig. 1).

In 73 groves a single sample was taken during the whole study period, 3 others were inspected each two weeks for 3 years and the remaining 12 groves were sampled monthly for a year*.

The groves sampled only once were visited mainly during summer time, which corresponds to the period where the greatest number of species might be captured (MAGRO, 1992, PAIXÃO, 1994). This is also the season where the key Homoptera are particularly abundant and we can so expect to find their predators more easily.

To collect ladybirds a method «Beating method» recommended by IOBC (AMARO & BAGGIOLINI, 1982) was adopted. 100 branches uniformly distributed in each orchard were selected and beaten with a stick. In order to standardise the sampling method, each branch was stricken three times. The insects so dislodged fell in a jar attached at the bottom of a tray in a form of a funnel. This method mainly yielded adult ladybirds but few larval instars.

Coccinellids were identified using keys published by CHAZEAU, *et al.* (1974), GOURREAU (1974), RAIMUNDO & ALVES (1986), FÜRSCH (1987 *a* and *b*) and RAIMUNDO (1992). Most identifications were achieved by considering external characters but there were still about 1.100 individuals for which extraction and observation of genitals were needed.

A geographical database was created with the help of *Microbanque Faune-Flore, logiciel de gestion de banques de données biogéographiques* (RASMONT, *et al.*, 1993). The same program allowed the drawing of distribution maps. Maps were plotted on a 10 Km square basis derived from the UTM grid maps.

A first analysis of the distribution was done using the dispersion index *ID* associated with a statistical test *d* (LUDWIG & REYNOLDS, 1988):

$$ID_i = \frac{s_i^2}{x_i} \quad d = \sqrt{2\chi^2 - \sqrt{2(N-1)}} - x_i$$

where \bar{x} , is the average number of ladybirds of species *i* in the orchard samples, s_i^2 is the estimate of the samples variance and *N* represents the total number of samples. If the samples follow a theoretical Poisson distribution, this ratio is expected to be equal to 1.0. Significant departures of *ID* from 1.0 can be tested using *d*. If $d < 1.96$, agreement with a Poisson distribution is accepted ($P > 0.05$). If $d < -1.96$, the ladybirds are suspected to be regularly dispersed among the orchards, and if $d > 1.96$, a clumped dispersion is likely (ELLIOT, 1973).

The study of distribution patterns was completed by SADIE, created by PERRY & HEWITT (1991) and ALSTON (1994). In the computations of measures and indices the program Sadiec.for** was used. This program analyses the spatial pattern of data that are in the form of counts at specified spatial locations. Techniques and notation follow those outlined in Perry (1995*b*).

The techniques measure distance to complete regularity/aggregation of the observed numbers and compare the result with those based on randomised simulations of the same data set. Tests and indices are so constructed.

Two types of simulation can be done:

* The periodical sampling meant to supply information to a second paper on temporal dynamics.

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Table 1. - Coccinellid species captured in citrus groves from the center/south of Portugal in 1991-94 and their food preferences (Fp). 1 = Diaspididae, 2 = Coccidae, 3 = Pseudococcidae, 4 = *Icerya purchasi*, 5 = Aphididae, 6 = mites, 7 = Aleyrodidae, 8 = plants, 9 = fungus, 10 = predators with unknown food preferences in citrus, (*) = species captured only in periodical sampling, Tp = total number of individuals captured in punctual samples.

TAXA	Fp	Tp
Chilocorini		
<i>Chilocorus bipustulatus</i> L.	1,2	158
<i>Exochomus nigromaculatus</i> (Goeze)	5	91
<i>E. quadripustulatus</i> L.	1,2,5	30
Coccidulini		
<i>Lindorus lophantae</i> (Blaisdell)	1,2	510
<i>Rhizobius chrysomeloides</i> (Herbst)	1	84
<i>R. litura</i> Fabricius	1	1
Coccinellini		
<i>Adalia bipunctata</i> (L.)	5	2
<i>A. decempunctata</i> (L.)	5	12
<i>Coccinella septempunctata</i> L.	5	8
<i>Oenopia conglobata</i> (L.)	5	20
<i>O. doublieri</i> (Mulsant) (*)	5	
<i>O. lyncea</i> (Oliv.) (*)	5	
<i>Propylea quatuordecimpunctata</i> (L.)	5	41
<i>Psyllobora vigintiduopunctata</i> (L.)	9	5
<i>Tytthaspis sedecimpunctata</i> (L.)	9	1
Epilachnini		
<i>Subcoccinella vigintiquatuorpunctata</i> L.	8	4
Hippodamiini		
<i>Hippodamia variegata</i> (Goeze)	5	5
Hyperaspini		
<i>Hyperaspis reppensis</i> Herbst	10	1
Noviini		
<i>Rodolia cardinalis</i> (Mulsant)	4	205
Platynaspini		
<i>Platynaspis luteorubra</i> Goeze	1	4
Scymnini		
<i>Clitosthetus arcuatus</i> (Rossi)	7	196
<i>Cryptolaemus montrouzieri</i> Mulsant	3	208
<i>Nephus bisignatus</i> Boheman	10	6
<i>Nephus includens</i> (Kirsch)	3	229
<i>Nephus reunioni</i> Fürsch	3	72
<i>Nephus ulbrichi</i> Fürsch	10	1
<i>Nephus binotatus</i> Brisout (*)	10	
<i>Nephus hiekei</i> Fürsch (*)	10	
<i>Nephus fuerschi</i> Plaza (*)	10	
<i>Scymnus mediterraneus</i> Khnzorian	10	1565
<i>Scymnus auritus</i> (Thunberg) (*)	10	
<i>Scymnus subvillosus</i> (Goeze)	10	140
<i>Scymnus suturalis</i> Thunberg	10	22
<i>Scymnus apetzi</i> Mulsant	10	86
<i>Scymnus interruptus</i> (Goeze)	10	503
<i>Scymnus levaillanti</i> Mulsant	10	14
<i>Scymnus rufipes</i> (Fabricius)	10	6
<i>Stethorus punctillum</i> (Weise)	6	150

- **a**: involves permutation of the actual counts observed amongst the sample units;
- **r**: involves totalling the counts over all the sample units and redistributing each individual of this total number, randomly, to the sample units.

In type **a** simulation inferences relate only to the spatial arrangement of the counts; in the type **r** simulation both spatial arrangement of the counts and the degree to which their distribution is non-Poisson are taken into account.

In the present work only tests and indices involving permutation (**a**) were used, for the information about the degree to which the distribution of counts is non-Poisson is already given and tested by the dispersion index *ID*. Only estimates based on distance to aggregation were analysed for the criteria of distance to regularity are sensible to edge effects (PERRY, 1995a).

As a result, the index J_a was calculated:

$$J_a = \frac{D}{E_a}$$

where *D* is the distance to aggregation of the observed samples and E_a , the average distance to aggregation for randomised samples.

The index J_a is followed by Q_a , which is the proportion of randomised samples with a distance to aggregation as large as, or larger than the observed value *D*.

For this computations the UTM co-ordinates of each grove, in a scale of 1 × 1 Km were taken and transformed to simple co-ordinates composed by two numbers corresponding to abscissae and ordinates.

Samples were grouped in 7 main regions (Fig. 1) and their Dominance (relative abundance - SZUJECKI, 1987) was calculated for each region. The Dominance of the different guilds of ladybird species in each region was also evaluated.

In the plotting of maps and analysis of distribution and dominance only data from the punctual sampling was considered. In the

case of groves subject to periodical sampling a single sample from summer time was chosen and used for the analysis mentioned above. Analysis of distribution patterns were done for species having at least 12 captured individuals in punctual samples, because at low population densities the distributions often approach the Poisson (MORRIS, 1960).

RESULTS

Table 1 presents the list of coccinellid species captured in citrus groves, their essential food when known and total number of individuals captured in punctual sampling.

38 species were identified. A few individuals of the species *Nephus peyerimhoffi* Sicard, claimed by RAIMUNDO (1992) as being present in Portugal, might be among those identified as *N. includens* which would raise the number of species to 39. We were not able to distinguish these two species due to the unclear descriptions of the genitalia of *N. peyerimhoffi* provided by RAIMUNDO (1992) and Fürsch (1987 b).

All species of the *Nephus* genus, with the exception of *N. 2-notatus* (i.e., 7 species) were described for Portugal only recently (MAGRO, 1992, RAIMUNDO, 1992, MAGRO, 1997, RAIMUNDO, pers. com.).

The identified species are divided into 9 tribes; 6 species appeared only in periodical sampling.

Several species are represented by 8 or less individuals.

Exception being made of *S. 24-punctata*, *P. 22-punctata* and *T. 16-punctata* all the coccinellids are predators.

In Table 2 we can find the results of the analysis of distribution patterns. In what the *ID* index is concerned, all species with the exception of *A. decempunctata* and *S. levaiillanti*, present significant variations in numbers from one sample to another (*ID* > 1). This spatial heterogeneity of the counts reveals an aggregated distribution but does not allow any inference about the spatial arrangement of those counts.

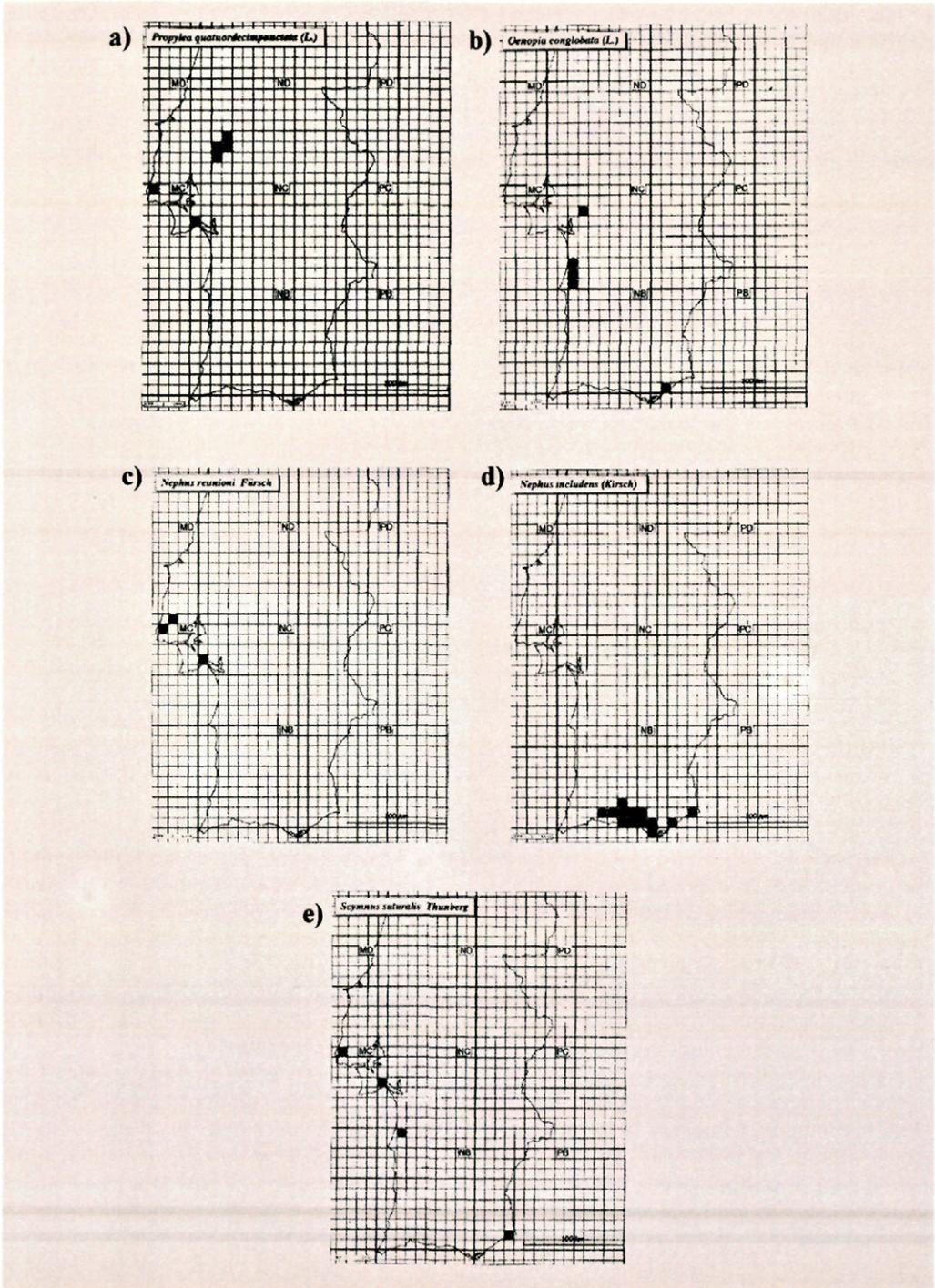


Fig. 2. - Distribution maps (UTM) of five species of coccinellids captured in citrus groves in Portugal.

Table 2. - Analysis of distribution patterns of coccinellid species present in citrus groves from the center south of Portugal. ID = dispersion index, Ja = aggregation index, d and Qa = statistical tests. ID or $Ja = 1$, random distribution; ID or $Ja > 1$, aggregated distribution; ID or $Ja < 1$, uniform distribution.

TAXA	ID	d	Ja	Qa
<i>Chilocorus 2-pustulatus</i>	9.5		0.9	>0.875
<i>Exochomus nigromaculatus</i>	9.9		0.9	0.625
<i>E. 4-pustulatus</i>	5.7		1.1	0.500
<i>Lindorus lophantae</i>	26.5		1.7	<0.125
<i>Rhizobius chrysoloides</i>	8.4		1.6	<0.125
<i>Adalia 10-punctata</i>	1.2	1.36	1.7	<0.125
<i>Oenopia conglobata</i>	5.7		6.6	<0.125
<i>Propylea 14-punctata</i>	10.0		2.6	<0.125
<i>Rodolia cardinalis</i>	6.1		1.0	0.500
<i>Clitosthetus arcuatus</i>	12.3		1.0	0.625
<i>Cryptolaemus montrouzieri</i>	42.4		0.7	0.875
<i>Nephus includens</i>	23.7		3.3	<0.125
<i>N. reunioni</i>	15.2		13.0	<0.125
<i>Scymnus mediterraneus</i>	193.8		1.3	<0.125
<i>S. subvillosus</i>	6.2		1.2	<0.125
<i>S. suturalis</i>	10.6		2.4	<0.125
<i>S. apetzi</i>	6.4		1.0	0.375
<i>S. interruptus</i>	32.1		1.0	0.625
<i>S. levaillanti</i>	1.1	0,93	1.0	0.375
<i>Stethorus punctillum</i>	8.9		0.8	>0.875

The results of the J_a index point out that half of the species have aggregated distributions of counts ($J_a > 1$); 5 of them - *P. 14-punctata*, *O. conglobata*, *N. reunioni*, *N. includens* and *S. suturalis* - show even very high values of that index. In the maps representing the distribution of those five species (Figure 2a - e) we can see that *P. 14-punctata*

has a strong presence in the Muge region, *O. conglobata* is mainly in the Santiago do Cacém region, *N. reunioni* was only caught in a 80 Km diameter region around Lisbon, *N. includens* was only captured in Algarve and *S. suturalis* was fundamentally restricted to the surroundings of Sintra/Mafra and Setúbal/Pegões. Nevertheless, the Q_a values do not indicate that any of those species presents a distribution significantly different from a Poisson one.

The remaining species have J_a values close to or below 1, with no significant departures from a Poisson distribution.

N. includens was, as said before, only recently described for Portugal. The possible evolution of the spatial pattern of distribution of this species in Algarve from 1991 to 1994 was evaluated with the help of SADIE (Table 3). No evolution was detected, counts having for the three years a Poisson distribution.

Table 3. - Analysis of the distribution pattern of *N. includens* in Algarve, for three years. ID = dispersion index, Ja = aggregation index and its statistical test, Qa . ID or $Ja = 1$, random distribution; ID or $Ja > 1$, aggregated distribution; ID or $Ja < 1$, uniform distribution.

Year	D	Ja	Qa
1991	15.3	1.134	0.500
1993	24.9	0.785	0.875
1994	10.0	1.038	0.375

Table 4. - The four most dominant species of coccinellids present in citrus groves from 7 regions in Portugal. Dominance values in %. DP = dominance position; N= total number of captured individuals.

DP	Regions							N
	Sintra/Marf a	Setúbal/Pegões	Muge	Santiago	Silves	Faro	V. R. Sto. António	
1 st	<i>L. lophantae</i>	<i>S. mediterraneus</i>	<i>C. montrouzieri</i>	<i>S. mediterraneus</i>	<i>N. includens</i>	<i>S. mediterraneus</i>	<i>S. mediterraneus</i>	
2 nd	(34) <i>S. interruptus</i> (16)	(31) <i>L. lophantae</i> (25)	(16) <i>L. lophantae</i> (16)	(20) <i>S. interruptus</i> (18)	(31) <i>C. arcuatus</i> (20)	(26) <i>N. includens</i> (19)	(65) <i>S. interruptus</i> (9)	
3 rd	(15) <i>R. cardinalis</i> (15)	(14) <i>S. interruptus</i> (14)	(12) <i>S. punctillum</i> (12)	(14) <i>C. arcuatus</i> (14)	(18) <i>S. mediterraneus</i> (18)	(13) <i>S. interruptus</i> (13)	(4) <i>L. lophantae</i> (4)	
4 th	(7) <i>S. mediterraneus</i> (7)	(7) <i>N. reunioni</i> (7)	(11) <i>S. interruptus</i> (11)	(13) <i>C. montrouzieri</i> (13)	(14) <i>S. subvillosum</i> (14)	(12) <i>C. montrouzieri</i> (12)	(3) <i>C. 2-pustulatus</i> (3)	
N	294	988	544	292	274	546	1.442	

Table 5. - Number of coccinellid species per guild and dominance values (%) in citrus groves from 7 regions in Portugal. Food preferences: scale insect (C), aphids (Ap), white flies (Wf), mites (Mi), fungus (Fu), Plants (Pl) and predators with unknown food preferences in citrus (?); N=total number of captured individuals.

Regions	Food preferences								N
	C	Ap	Wf	Mi	Fu	Pl	?		
Sintra/Mafra	6 (53)	2 (4)	1 (6)	1 (2)	-	-	6 (35)		294
Setúbal/Pegões	8 (45)	5 (2)	1 (3)	1 (1)	-	-	9 (49)		988
Muge	6 (49)	5 (15)	1 (2)	1 (12)	-	-	5 (22)		544
Santiago	8 (29)	4 (9)	1 (14)	-	-	1 (1)	8 (48)		292
Silves	6 (40)	2 (1)	1 (20)	1 (2)	-	-	5 (37)		274
Faro	6 (43)	2 (1)	1 (3)	1 (7)	-	-	7 (47)		546
V. R. Sto. António	8 (14)	5 (2)	1 (2)	1 (2)	1 (<1)	-	8 (79)		1.442

Table 4 shows the four most dominant species in each region. Of the original 38 species only 11 occupy these main dominant positions. *S. mediterraneus*, *S. interruptus* and *L. lophantae* are the most common.

Among the 11 referred species, 6 are coccidophagous, 3 have unknown predatory regimes in citrus and the remaining species feed respectively on white flies and mites.

S. mediterraneus and *S. interruptus* are dominant in 6 of the 7 considered regions.

In what guilds are concerned (Table 5), coccidophagous species and species which food preferences are unknown in citrus are dominant. Aphidophagous are, in general, badly represented, *E. nigromaculatus* being the most abundant (Table 1).

DISCUSSION AND CONCLUSIONS

The results presented in Table 1 confirm that coccinellids are the most important group among citrus predators in Portugal: 36 species against only 12 species of Chrisopids (Chrisopidae: Neuroptera) (Pantaleão, *et al.*, 1994), 7 of Coniopterigids (Coniopterygidae: Neuroptera) (CARVALHO & FRANCO, 1994), 3 of Anthocorids (Anthocoridae: Heteroptera) and 4 of Mirids (Miridae: Heteroptera) (SILVA, *et al.*, 1994).

The identified species correspond to 61% of the total coccinellids referred to Portugal by RAIMUNDO & ALVES (1986), RAIMUNDO (1992) and RAIMUNDO (*pers. com.*). The Scymnini tribe is the best represented.

The species mentioned as being represented by 8 or less individuals are probably either rare species or species that do not use citrus as a typical habitat (HODEK, 1973).

The aggregated distributions observed for almost all species (*ID* index values, Table 2) are not surprising for, as RABINOWITZ (1981) refers, changes of orders of magnitude in population sizes occur on the scale of meters without striking underlying heterogeneity. In fact, the number of abiotic and biotic conditions that can influence population numbers are huge. In agroecosystems the constant

human interventions add themselves to the disturbing factors.

The random distributions of *A. 10-punctata* and *S. levaillanti* might be the result of the small number of captured individuals, as defended by MORRIS (1960). In fact the two species were included in the analysis though the number of captured individuals was still low (12 and 14 respectively, against 20 for *O. conglobata* the next more abundant species).

In what distribution of counts are concerned (*Ja* values, Table 2), the fact that all species distributions are not significantly different from a Poisson might be the result of the sampling strategy; sampling effort was not uniform and samples were collected in a rather discontinuous way. It is nevertheless possible to say that SADIE results point out the tendency of some species for an aggregated distribution. Among those species *N. reunioni* and *N. includens* are quite abundant.

N. reunioni was first described in 1974 for the Reunion island (CHAZEAU, *et al.*, 1974) and as been since then introduced in several countries for the biological control of mealybugs, namely *P. citri* in citrus. In Portugal this coccinellid was first detected in 1990 in the Setúbal region. Its presence might be the result of the introduction of some individuals in a citrus grove at Oeiras (near Lisbon) in 1984 (Magro *et al.*, 1992).

As we referred to, *N. reunioni* was restricted to an 80 Km region around Lisbon but it might be spreading, with our sampling method being unable to detect newly established small populations. *N. includens* is a mediterranean species, the first individuals of which were captured in Portugal (Algarve) in 1984 (MAGRO, *et al.*, 1992). The results presented in Table 3 make it possible to conclude that the interval of time between the introduction of the species and 1991 is long enough to allow a random pattern of distribution of individuals in the all Algarve region.

TRANFAGLIA & VIGGIANI (1972/73) claimed that this coccinellid presents biological characteristics similar to *C. montrouzieri*, which leads us to think that its restriction to Algarve is mainly due to geographical

barriers not allowing its progression to the North. The fact that this coccinellid has quite a narrow range of food can also be in the origin of this restriction to Algarve; the region immediately north of Algarve might not present the adequate conditions to its survival.

An interesting fact is that *R. cardinalis* and *C. montrouzieri*, exotic coccinellids introduced in Portugal in 1888 and 1918 respectively for the biological control of *I. purchasi* and pseudococcids, can now be found in all the citrus regions.

Although the taxocenoses structure is variable among regions (Table 4 and 5), a basic pattern seems to emerge, dominance main positions and dispersion belong to a group of species among which we can find well known predators of citrus important pests.

The important place occupied by species which food preferences are unknown in general and in citrus in particular is quite interesting. All those species, belonging to the Scymnini tribe, can be potential predators of pseudococcids, at least in the adult stage, but no specific studies were done until now (MAGRO, 1992).

The overall small representation of aphidophagous species might be due to the fact that summer time is not favourable to their presence in citrus.

The present work shows the extreme richness of the coccinellid fauna of citrus in Portugal. In this country the primary biotic and abiotic conditions for the activity of those species seem to be present. The fact that exotic species acclimatise and spread without programmed introductions is very significant. This situation contrasts greatly with what has been observed for the countries of the close mediterranean region (e.g. LONGO & BENFATTO, 1987).

The captured predators cover an all array of preys, coccidophagous species being the best represented. This is quite important as several coccids attack citrus. It is however well known that the action of the natural

population of coccid enemies is not sufficient to keep some of those pests under the economical thresholds; it is the case in Portugal for *P. citri*, *S. oleae* and *L. beckii*. Either the auxiliaries are not effective or their action is limited by chemical treatments. In what effectiveness is concerned, MERLIN, *et. al.* (1996*ab*) have shown that *C. montrouzieri* is able to assess the quality of coccid colonies and change their oviposition decision accordingly. In the presence of larvae of their own species that coccinellid can withhold eggs and leave the colony. Identical results had been previously found for *Adalia 2-punctata* (Hemptinne, *et. al.*, 1992). These results point out the need to complete natural control by the introduction of larvae of these predators.

In the second case, the use of specific pesticides only when economical thresholds are attained would possibly reduce greatly the level of those phytophagous and other pests in general.

The abundant group of coccinellids which food preferences are unknown in citrus might constitute an amazingly important potential for biological control in this crop. It would be important to investigate their role, particularly in the case of *S. mediterraneus* and *S. interruptus*.

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