Since we are here talking about new species, our further research will by all means go in the direction of researching their biology, as well as their determination on the host plants.

Conclusion

In the course of a three-year faunistic research, 24 species have appeared as the newly established species of aphids for which a site in Croatia has been found for the first time. Out of 24 species, 11 species of aphids were determined by Igrc Barčić in the course of 80's (the determinations have not been published), while the same species have been established during this research as well. 13 species of aphids have been determined for the first time in Croatia (Gotlin Čuljak).

Given the importance of aphids, as well as their numerosity (there are around 4500 species worldwide, and 199 known in Croatia), we feel that our faunistic research should be both continued and broadened.

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COMPARISON OF *MAYETIOLA* SPECIES ASSEMBLAGES AT DIFFERENT GEOGRAPHICAL SCALES: INFLUENCE OF HOST PLANT VARIATION

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In Tunisia, two species of *Mayetiola* (*M. destructor* and *M. hordei*) have been identified on the basis of diagnostic alleles and PCR-RFLP of the cytochrome b gene. They are the major pests of wheat and barley responsible for serious crop losses. As these two species show feeding preferences between cereals, knowledge of the population dynamics of this phytophagous insect is needed in order to survey ecology and evolution of host parasite interaction. In this study, the PCR-RFLP technique based on the cytochrome b gene was used to identify the two species of *Mayetiola* and to investigate the relationships with their hosts. The comparison of *Mayetiola* assemblages on barley in different regions in Tunisia, showed a continual geographical gradient variation in species compositions and dominance order. However, on wheat there is a homogenous distribution. Thus, monitoring of pest must take into account our finding that population assemblage differs from host types and geographical location.

Diptera, Cecidomyiidae, *Mayetiola*, Tunisie

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U Tunisu su utvrđene dvije vrste roda *Mayetiola (M. destructor i M. hordei)* koristeći diagnostičke allele i PCR-RFLP citokroma b gena. Ove su vrste važni štetnici pšenice i ječma te čine velike štete. Kako pokazuju preferenciju pri ishrani ovih žitarica potrebno je poznavati njihovu dinamiku populacije kako bi se mogle pratiti

interakcije između štetnika i domaćina. U ovim istraživanjima korištene su PCR-RFLP tehnike temeljene na citokromu b gena za identifikaciju obih vrsta i utvrđivanje odnosa prema domaćinu. Usporedba skupina vrsta roda *Mayetiola* na ječmu u različitim dijelovima Tunisa pokazala je kontinuirani geografski gradijent u sastavu vrste i redu dominantnosti. Međutim, na pšenici je distribucija homogena. Stoga se kod monitoringa štetnika moraju uzeti u obzir ovi nalazi prema kojima populacija ovisi o vrsti domaćina i geografskog lokaliteta.

Diptera, Cecidomyiidae, *Mayetiola*, Tunis

Introduction

The Hessian Fly is a ravagers of many agricultural crops. Damage to host plants occurs primarily by growth loss that results from adult tunnelling and feeding on the stem which lend to break and fall over. The infested plants are characterized by a decrease in the number and the size of the seeds, a reduced elongation of the 2^{end} leaf, a complete stunting of the 3^{rd} leaf and a prevention of further leaf development or initiation (CARTWRIGHT, 1959).

In Tunisia, midges of the genus *Mayetiola* are a serious plague of cereals. Crop losses are caused by two species of *Mayetiola* (*M. destructor* and *M. hordei*) which have been identified on the basis of diagnostic alleles (MAKNI et al, 2000a). A recent study, based on the PCR-RFLP of the cytochrome b gene, has shown that *M. destructor* is represented by only one mitochondrial haplotype designed by A and *Mayetiola hordei* is characterized by two mitochondrial haplotypes designed by B and C (MEZGHANI et al., 2002 a, b). In addition, as previously shown (MAKNI et al, 2000b), there was no strict association between the cereal species (wheat and barley) and the *Mayetiola* species.

Surveys of these insect infestations have shown that the rate of infestation is higher on barley than on wheat. Indeed, in the very attacked zones infestation could reach 90% on barley against 50% on wheat (MILLER et al., 1989). In order to develop the integrated pest management (IPM) programs of cecidomyies, it is essential to know the distribution of each *Mayetiola* species beyond their host. In fact, it has been established that female phytophagous insect should oviposit where their offspring have the greatest probability of survival (CRAIG et al., 1989; COURTNEY & KIBOTA, 1990; PRICE, 1991; OHGUSHI, 1995; CRAIG et al., 2000). Many factors can influence oviposition preference and offspring performance in phytophageous insects as plant genotype (CRAIG et al., 1989; LARSSON & STRONG, 1992; LARSSON et al., 1995; PRESZLER & PRICE, 1995), plant vigour (PRICE et al., 1990; PRICE, 1994) and intraspecific competition (WHITHAM, 1980; PROKOPY et al., 1984; CRAIG et al 1989).

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Moreover, the distribution of an insect species across a set of host plants at the landscape level is influenced not only by spatial variation in environmental conditions or resource distribution (DENNO and MCCLURE 1983, VIA 1994, MOPPER and STRAUSS 1998) but also in the spatial variation in host plant abundance (WIKLUND 1974, COURTNEY and FORESBERG 1988). Studies of multiple factors can help to clarify the underlying causes of oviposition preference and offspring performance between correlated factors.

In this paper we investigated the interaction between *Mayetiola* species (*M. destructor* and *M. hordei*) and their hosts (wheat and barley) in order to know if populations of *Mayetiola* differentiated or do all insect from all host plant species belong to a single genetic community and also if isolating mechanisms related to host association result in assortative mating of similar host feeding populations. We have also analysed the separate effects of the rainfall and the host abundance on the population dynamics of *Mayetiola*.

Natural history

The herbivorous insects *Mayetiola destructor* and *M. hordei* are Diptera Nemotcera belonging to the Cecidomyiidae family. SKUHRAVA et al (1984) have described the life history of *M. destructor*, there are usually two generations of this species per year. Adults emerge in February to late March and after mating females lay their eggs on the upper surface of leaves of cereals. The larvae of the first generation occur during February and March. The larvae feed by sucking the sap from the tissues of the stems. They develop on their host plants for about 18-30 days. On completing their feeding and growth, their upper skin hardens and turns brown and the larvae itself shrinks away from this outer brown cover, the puparium. The third stage larva remains inside the puparium without feeding for a variable period. Inside the puparium the larva changes into a short prepupal stage followed by pupation within the puparium on the stem of the host plant during the middle of July. Adults emerge in autumn and produce the second generation, they live approximately 3-5 days.

Methods

Insect material

We collected from Tunisia 396 individuals insects on barely representing 31 populations and 128 on wheat representing 7 populations. Collection was made from January to June on 1999 and 2000 (Tabl. 1). Populations were coded using numbers. Infested plants from each field were placed in separate screened cages in a greenhouse where conditions were favourable for adult emergence (12:12h photoperiod, 18 ^{o}C-20

°C temperature and 60% relative humidity). Upon emergence, adults were removed from the cages and stored in 1.5 ml microcentrifuge tubes at –70 °C.

Mitochondrial DNA PCR

For PCR DNA template, we used the individual insect DNA extractions of DOYLE & DOYLE et al. 1987 by grinding them with a piston in a 1.5 ml microcentrifuge tube in 50 µl extraction buffer (2% CTAB; 1.4 M NaCl ; 0.2% 2-mercaptoethanol, 20 Mm EDTA ; 100 mM Tris-HCl; pH 8). The pestle was washed with an additional 150µl of extraction buffer, the tube capped and then incubated at 65 °C for one hour. The homogenate were gently mixed with 1 volume of chloroform for 5' and after 10 minutes the aqueous phase was collected by centrifugation at 6000 g. One volume of isopropanol were added and kept at –20 °C for one hour. The DNA pellets were obtained by centrifugation at 13000g (15'), rinsed in 70% ethanol, air dried, and suspended in 20µl of sterile water.

Amplification

A 800 pb of the cytochrome b (Cyt b) gene was amplified using the primer CP1 and CP2 (Harry *et al.*, 1998). PCR reaction mixtures contained 10X Taq DNA polymerase buffer 3mM MgCl2, 0.1 mM dNTP 1U of Taq polymerase (Promega) 0.7µM of each primer (forward: 5' GAT-GAT-GAA-ATT-TTG-GAT-C 3' and reverse: 5' CTA-ATG-CAA-TAA-CTC-CTC 3'). Final reaction volume were 25µl including 5µl of DNA template. Cycling conditions consisted of: one cycle at 95 °C (3') 34 cycles at 95 °C (1') 48 °C (1'30"), 72 °C (1'30") and one cycle at 72 °C (10').

About 7 to 10µl of the PCR product were digested with the restriction endonuclease *MseI* and separated in 8% acrylamid gel. The gel was stained with ethidium bromide and visualized under UV light.

Statistical analysis

We subjected the data on relative abundance of the two species counts to multivariate analysis. The choice of appropriate statistical methods was dictated by the need to obtain a multivariate indicator that would show the distribution of each species on the two host types.

In a situation such as this, where there is an a priori group structure on barley, the factor analysis of correspondence (FAC) is considered most appropriate (BENZECRI, 1973). It was used to compare insect species richness among the two host plants. Each haplotype is considered as a character. FAC identifies several independent axes, which account the greater part of the variation. These axes are linear combinations of the characters and each character can be located along them. Each population was characterized by the contribution of each character (haplotype) to the two first axes and

distinctive population or population groups were separated and re-analysed to recognize another variation pattern.

Table 1. Haplotypes frequencies in different studied sites

Host		Population (N*)	Code	% A	% B	% C
Barley	North East	Sidi Boussaid (13)	n1	0	23,076	0,769
		Raued (10)	2	20	10	70
		Utique (12)	3	0	25	75
		Zaoia (8)	4	0	12.5	87.5
		Essaida(8)	5	0	12.5	87.5
		Mateur(8)	6	25	25	50
		Djbel oust (6)	7	0	0	100
		Ain askar (4)	8	0	0	100
		Gadhouna (10)	9	0	10	90
		Kobet lagna (16)	10	0	31.25	68.75
		Korba (6)	11	0	16.66	83.33
	North Ouest	Fahs (11)	12	0	45.5	54.5
		Goubellat (10)	13	0	10	90
		Goubellat (7)	14	0	42.8	57.1
		Ain tonga (22)	15	0	18.18	81.81
		Teborsouk (10)	16	0	20	80
		Douggua (7)	17	0	14.285	85.71
		Oued zargua (17)	18	23.52	5.88	70.588
		Borj Masoudi (12)	19	8.33	25	66.66
		Zahfrane (7)	20	0	42.85	57.14
		Sers (22)	21	0	36.36	63.63
		Zaouarine (17)	22	0	35.29	64.7
		Dogua (7)	23	0	34.15	65.85
		Dahmani (26)	24	0	42.3	57.69
		Zaouarine (7)	25	0	57.14	42.85
		Siliana (4)	26	0	50	50
		Fahs (11119)	27	0	36.84	63.15
	Center	Ain jaloula (10)	28	0	50	50
		Kairouan (24)	29	0	41.66	58.33
	South	Tina (15)	30	0	60	40
		Eljem (4)	31	0	75	25
Wheat	North East	Djebel oust (10)	32	53.84	7.6	38.46
		Chouat (24)	33	33.33	8.33	58.33
	North Ouest	Testour (11)	34	81.81	9.09	9.09
		Beja (7)	35	85.71	14.28	0
		Goubellat (15)	36	60	13.33	26.66
		Krib (10)	37	80	20	0
	South	Sidi Abid (16)	38	62.12	10.33	27.55

N* - number of individuals analysed per population

The SAS statistical analysis package (SAS System for windows, SAS institute, Cary, NC) was used for these and all other statistical analyses.

Results

A total of 422 individuals were collected, the PCR-RFLP analysis allowed us to distinguish between the two species and to determine their haplotype (Tabl. 1). We calculated the proportion of each haplotype and we applied FAC on the relative abundance of two species of Mayetiola on each host. We sought a measure that reflected the site-to-site difference in cecidomyie composition (assemblage structure).

Fig. 1. Geographical cline of haplotypes distribution on barley

Mayetiola abundance:

Two clear patterns emerged from examination of the abundance of each species in the two different hosts. *M. hordei* is present on barley as well as on wheat whereas *M. destructor* is restricted to wheat with some local exceptions (n6, n18 and n19). Table 1 shows the proportions of the three haplotypes in terms of host plant species.

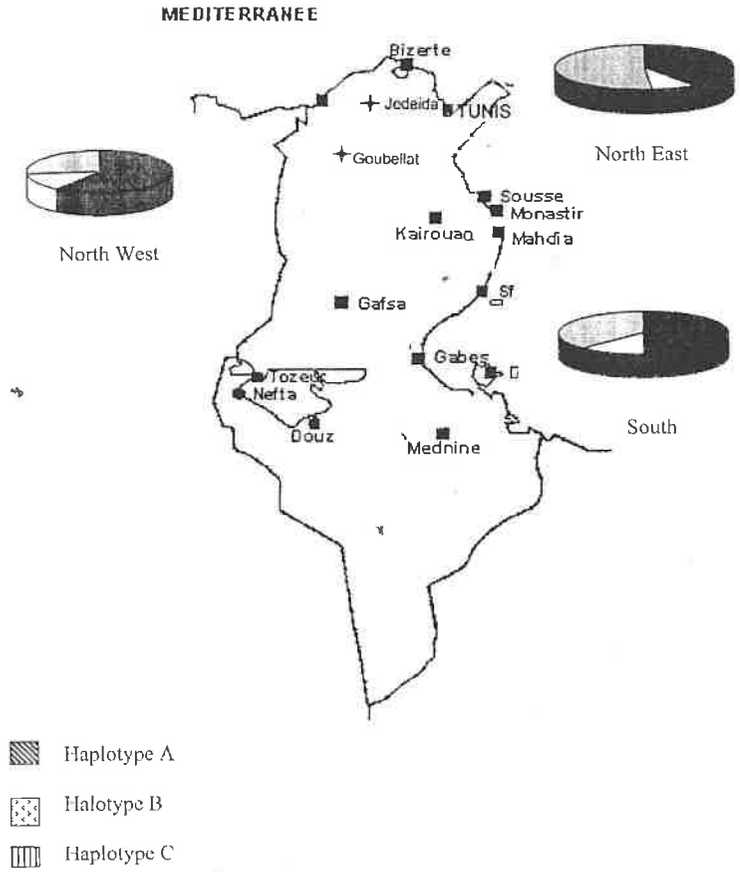

Fig. 2. Homogenate distribution of the three haplotypes on wheat

On barley, there is a geographical cline in that the abundance of *M. hordei* of C haplotype declines from North to South however *M. hordei* of B haplotype, increase in number from the South to the North, this was well illustrated by Figure 1. In fact, populations from the northern regions are characterized by principally the C haplotype

(65.7 %-80.8%), those from the Centre by the two equally (45.8%-54.2%) and southern populations by the B haplotype (67.5%). However, *M. destructor* is present with lower abundance levels on the northern regions (16.5% %) and it is absent on the south.

On wheat, there is a homogenate distribution of the different haplotypes in that no clear pattern has been shown: *Mayetiola destructor* (haplotype A) is the most common (33.33%-81.81%) followed by the *M. hordei* of C haplotype (9.1%-58.33%) then *M. hordei* of B haplotype (8.33%-13.33%) (Fig.2).

It is important to mention that on both years, frequencies of the three haplotypes remain unchanged throughout the different studied sites (data not shown).

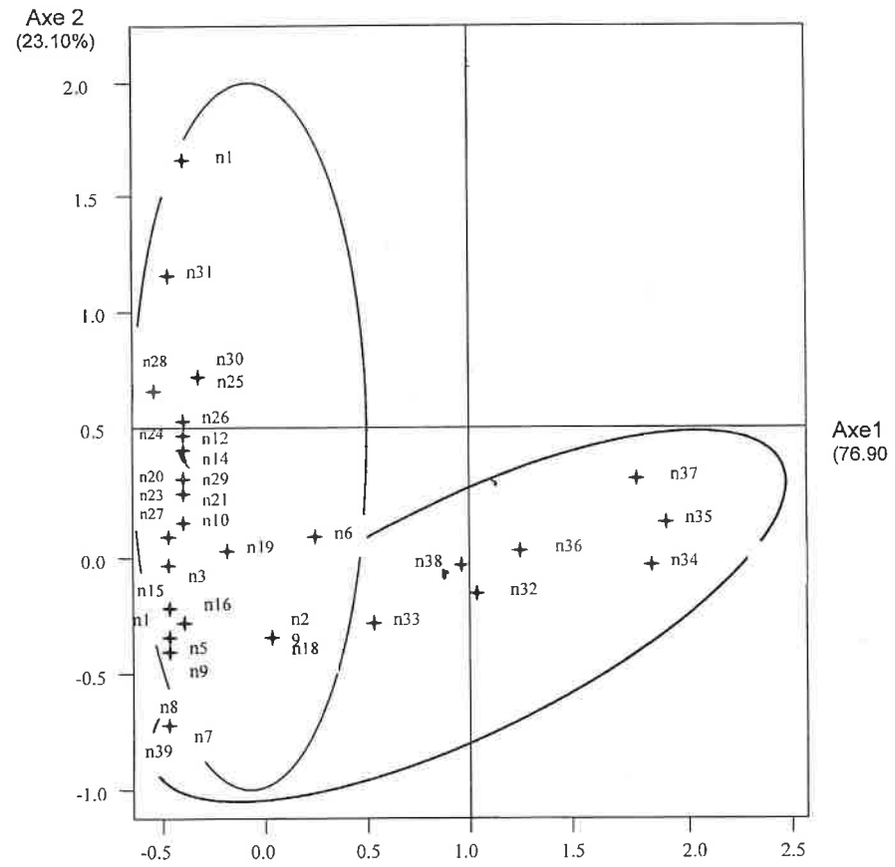

Fig. 3. Factorial analysis of correspondence (F.A.C.) on population of Mayetiola, done on the haplotype frequencies

2) Variance analysis

Each population was the subject of an analysis of variance against the treatment variables percentage of haplotype. Study of Mayetiola species organisation was carried out using the factorial analysis correspondence. Analysis was run with all the haplotypes as active variables. The results have shown that the two first axes explain 100% of the total variability. FAC axe 1 and axe 2 carry 76.58% 23.32%, respectively. The simultaneous projection of the population and the variables revealed a great differentiation in cecidomyie composition and shows well defined structuring of Mayetiola species in relation to their geographical origin and their host (Fig.3).

Not surprisingly there was considerable congruence between the outcomes of the FAC and the graphical analyses.

3) Influence of vegetation species abundance

Vegetation species composition change gradually with geographical position: in the northern regions, the surfaces of wheat sown are superior to those of barley (73.3% and 26.7% respectively). However, on the southern regions, the surface of barley dominate those of wheat (61.71% and 38.29% respectively).

Our results indicate that when the surface of wheat sown is superior to those of barley, *M. destructor* reproduce essentially on wheat and it could be found on barely with low frequencies. However, *M. hordei*, of C haplotype deposit their eggs on wheat as well as on barely .

When the surfaces of barley dominate those of wheat, *M. destructor* is found exclusively on wheat; *M. hordei* of B hapotype is more common than *M. hordei* of C haplotype on barley.

4) climatic factors

The most important climatic factor appears to be rainfall, in fact in the regions where precipitation is under 260 mm *Mayetiola destructor* is found exclusively on wheat and it is absent on barley. On the other hand, *M. hordei* of C haplotype is abundant comparing to B haplotype on wheat as well as on barley. However, in regions where rainfall is over 260 mm, *Mayetiola destructor* is found on barley with frequencies reaching 4 and 8% and *Mayetiola hordei* of B haplotype is abundant. On wheat, frequencies of C haplotype are superior to the B haplotype.

Discusion

The analyses by PCR-RFLP of the population of cecidomyies have permitted us to distinguish between the two species of Mayetiola. Both graphical and FAC analyses have shown a different spatial pattern of distribution of cecidomyies on the two cereals.

On barley, Mayetiola species richness changes with geographical position, mainly along a north-south axis. In the Northern part of Tunisia, The *M. hordei* of C haplotype is relatively abundant, falling further south in detriment of B haplotype, which increase toward the southern regions. The M. destructor is present only on the northern region with lower proportion.

On wheat, there is a homogenous distribution of the three haplotypes and the two species of the phytophageous insect co-occur but we notice that *M. hordei* is well represented by the C haplotype rather than the B haplotype.

Such selective distribution may be explained by geographical position: in the continental parts of northern Tunisia where cold and wet conditions predominate, the number of *M. hordei* of C haplotype is relatively high on barley as well as on wheat but in the southern regions where hot and dry weather predominates, female of *M. hordei* of C haplotype prefer to reproduce on wheat rather than on barley.

SKUHRAVA (1986) based on long term research on the distribution of gall midges in Europe came to conclusion that gall midge species richness is influenced by the following factors: geographical and altitudinal position (both of which are associated with changing levels of climatic variables including temperature and rainfall) and by the type of host. Our studies have shown that the host abundance could also influence the *Mayetiola* distribution.

Biological control is beneficial because it avoids the undesirable effects of chemical controls, which can results in the build up of persistent chemicals in the environment or the development of pesticide resistance. However, such approach needs the comprehension of the relationship of the cecidomyie-host system to develop successful biological control strategies.

Our study highlights several implications for pest management because the *Mayetiola* assemblage is basically determined by geographical location on barley. So pest surveys towards *Mayetiola hordei* must be intensive against the C haplotype in the northern regions and against the B haplotype in the southern regions. However, on wheat monitoring of the pest should be against *M. destructor* as well as *M. hordei* belonging to the C haplotype.

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MASS REARING OF *RHYNOCORIS MARGINATUS* FAB. ON LIVE AND FROZEN LARVAE OF *CORCYRA CEPHALONICA* STAINTON

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Rhynocoris marginatus (Fab.) is a predator of larvae and nymphs of many pestiferous insects for which natural enemies are not commercially available. This paper describes a containerised laboratory mass-rearing method for *R. marginatus* using refrigerated-killed larvae of *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae). Cold-killed larvae prolong the nymphal developmental period; reduce the fecundity and hatchability, net reproductive rate and pre-oviposition time. The rearing method avoids the need for live insect prey, reduce the predatory rate and labour efficient. Frozen larvae had no impact on female body weight, innate capacity for increase in number, corrected generation time, weekly multiplication and doubling time. Cold-killed *C. cephalonica* larvae provided predator oviposit latter and laid eggs for many days and also reduce the nymphal mortality. Predatory behaviour studies show that this predator predates both alive and cold-killed preys.

***Rhynocoris marginatus*, laboratory rearing, *Corcyra cephalonica*, frozen larvae, biology, fecundity, predatory behaviour, life table**

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Rhynocoris marginatus F. je predator ličinki i kukuljica mnogih štetnih kukaca za čije suzbijanje nema komercijalno raspoloživih prirodnih neprijatelja. Ovaj rad opisuje metodu laboratorijskog masovnog uzgoja koristeći smrzavanjem ubijene ličinke vrste *Corcyra cephalonica* Stainton (Lep.: Pyralidae). Hladnoćom ubijene ličinke produljuju razdoblje razvoja kukuljica, smanjuju plodnost i postotak izlaska iz jaja, neto reproduktivni omjer i vrijeme preovipozicije. Metoda izbjegava potrebu za živim domaćinom, smanjuje omjer predatorstva i radno vrijeme. Smrznute ličinke ne utječu na težinu ženki, sposobnost povećanja brojnosti, trajanje generacija, tjednu multiplikaciju i vrijeme podvostručenja populacije, produljuju vrijeme ovipozicije i smanjuju smrtnost kukuljica. Istraživanja ponašanja ukazuju da ova predatorska vrsta napada živog i hladnoćom ubijenog domaćina.

***Rhynocoris marginatus*, laboratorijski uzgoj, *Corcyra cephalonica*, smrznute ličinke, biologija, plodnost, ponašanje predatora**