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Species diversity and distribution patterns of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) from various agricultural crops in Tunisia

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Abstract
The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a worldwide pest considered to be a complex of cryptic species. Until 2010, the *B. tabaci* complex comprised 24 distinct putative species. Recently, at least 15 new species have been reported. The objective of this study was to identify *B. tabaci* species present in different vegetable crops and on two ornamental plants in Tunisia. The genetic identity of 146 whitefly populations was determined by PCR-RFLP analysis of the COI gene and revealed that only *MEAM1* and *MED* species have been found in Tunisia. Among them, *MEAM1* species was dominant in 96 populations (100.0%), *MED* was dominant in 34 populations (100.0%) and the 16 remaining were a mixed populations. Species differentiation between localities was not significant, however, strong differences in the species dynamics were recorded according to host plants type. Thus, the *MEAM1* species was resulted largely predominant on vegetable crops (86.7%), whereas *MED* was consistently found on ornamental plants (97.3%).

Keywords: *B. tabaci*, distribution, host plants, MEAM1 and MED species, Tunisia.

1. Introduction
Invasive pest species have large impacts on agricultural crop yields, and understanding their genetic diversity will advance our knowledge about its population ecology, which can be important for developing effective management strategies [1]. The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is one of the most invasive and damaging agricultural pests worldwide. Highly polyphagous, it causes damage in various agricultural crops either directly by sucking phloem sap or indirectly by excreting honeydew onto the surface of leaves and fruits [2]. Furthermore, *B. tabaci* is a major vector of numerous plant viruses to a wide range of economically important crops including ornamental and vegetable plants that are responsible for severe crop losses. It is known to transmit over 100 plant viruses in the families' *Geminiviridae*, *Closteroviridae* and *Potyviridae* including *Tomato yellow leaf curl virus* (TYLCV) [3], which is the most limiting factor afflicting tomato production in Tunisia [4].
B. tabaci has been considered a species complex consisting of many biotypes and/or host races that differ greatly in host range, insecticide resistance, and ability to transmit plant viruses [5]. A recent phylogenetic analysis suggested that *B. tabaci* is a complex of 11 well–defined, high–level groups containing at least 24 morphologically indistinguishable putative/cryptic species [6, 7]. The two most widespread putative species are referred to *as Middle East–Asia Minor1* (*MEAM1*; *B* biotype) and *Mediterranean* (*MED*; *Q* biotype), both of which are invasive pests throughout the world [8, 9]. The damaging *MEAM1* species is defined by high fitness parameters such as high fecundity and fertility and it is an effective vector of viruses [10], whereas the *MED* species is associated with outbreaks of severe insecticide resistance and have developed resistance to all major insecticide groups [11].
Life–history traits such as resource exploitation and resistance to insecticides may affect the distribution and frequency of the different members of the *B. tabaci* species complex [11]. In agricultural areas, human activities, including cultivation practices, the use of cultivated plants and pesticide treatments, create an intense selection pressure on populations and may have a major influence on population demographics and spatial distribution patterns [12, 13]. In Tunisia, the first sighting of the *B. tabaci* species complex has never been directly recorded. On the other hand, the first report of *Tomato yellow leaf curl* disease, a *B. tabaci* transmitted virus disease was in the 1980s [14]. However, it is now very widely distributed and has assumed

primary pest status on several vegetable crops (especially *Cucurbitaceous* and *Solanaceous* species) as well as ornamental crops and its control is based mainly on chemical insecticides. In both greenhouse and open field crops, difficulties in controlling the pest have been reported, raising substantial concerns on the efficacy of the applied chemistries [15]. The identification of *B. tabaci* species is an important task, given the impact that they have on crop yields. Recently, a few research papers dealing with the *B. tabaci* complex have been published [15, 16, 17, 18] and until now, 2 tentative species, *Middle East–Asia Minor1* (*MEAM1*) and *Mediterranean* (*MED*), have been recorded in Tunisia. These species were namely identified based on mitochondrial cytochrome oxidase I (mtDNA COI) sequences and/or polymerase chain reaction (PCR) restriction–fragment–length polymorphism (*TaqI*). However, studies on the host range and distribution of *B. tabaci* species are limited and no large–scale survey is available for all of Tunisia. These previous studies involved few collection sites and only some vegetables (*Solanaceous* species) and ornamental (lantana, *lantana camara*) crops. As a result, information is not sufficient to understand the current status of the *B. tabaci* complex in Tunisia. On the other hand, the number of invasive alien species has continuously increased in Tunisia because of increased global trade and developments in transportation. Thus, possibly, other species of the *B. tabaci* complex may have invaded Tunisia. Currently, several species of the *B. tabaci* complex, such as the *MED, MEAM1*, *T*, *M* or *S* species as delineated by Dinsdale *et al.* [6] are known to be present in the Mediterranean basin [8, 15, 19, 20]. For this, studies investigating Tunisian population patterns of *B. tabaci* on their major hosts in agro-ecological conditions through time are needed to better understand host-plant utilization and its potential implications for population management.

Therefore, the objective of this study was to investigate the current status of the species composition of *B. tabaci* associated to a large panel of host plants in six localities in Tunisia covering north, central and south of the country. We testing the hypothesis that geographical and/or host plant components (as a specie, as a family or as a host type) are significant factors underlying the distribution patterns of *B.tabaci* species. To that end, an extensive survey covering an entire region of the whitefly habitat in Tunisia was conducted between 2009 and 2013. Using restriction fragment length polymorphism analysis of polymerase-chain-reaction-amplified fragments (PCR-RFLP) of the COI gene [21, 22, 23, 24], we determined the species composition in 146 whitefly populations and their distribution according to host plant and across six regions. On the other hand, the results were compared with published data of the *B. tabaci* complex to assess if changes in the genetic diversity of *B. tabaci* have occurred during the last years and discuss if this distribution can be correlated with the chemical treatments used in management program against *B. tabaci* populations and other pests.

2. Material and Methods

2.1. *B. tabaci* sampling

Sampling was conducted from Mars 2009 to December 2013 throughout six major vegetable producing localities in Tunisia; the extreme north and the north–eastern of Tunisia including Bizerte and Korba, the eastern part of central and central including Monastir and Kairouan, the southern part of Tunisia including Gabes and Kebili (Fig. 1). Samples were collected from eight vegetable crops (potato, *Solanum tuberosum*; tomato, *Solanum lycopersicum*; pepper, *Capsicum annuum* ; aubergine, *S. melongena*; bean, *Phaseolus vulgaris*; melon, *Cucumis melo* ; courgette, *Cucurbita pepo* and cucumber, *Cucumis sativus*) and tow ornamentals plants (*Hibiscus mutabilis* and lantana, *Lantana camara*), which has become a dominant component of natural and agricultural ecosystems. The whiteflies were collected from plants into glass Pasteur pipettes attached to a hand–held aspirator. Each collected population in each location was collected from different leaves on different plants and were kept in vials with 90% ethanol at –20°C until DNA extraction. As *B. tabaci* is a haplodiploid species, only females were kept for DNA extraction. In total, 146 populations were collected and for each population 10 to 20 individuals were used for molecular analyses. One population consisted of individuals of *B. tabaci* collected from several individual plants of the same host–plant species growing in a restricted site (i.e., a field or a greenhouse production system, from nearby vegetable crops or in public garden in the case of *L. camara and H. mutabilis*). Collection details, geographical locations, host plants and dates of collection are summarized in Table 1. Whitefly populations on greenhouse crops and some open fields were subjected to insecticide treatments, while the populations on *L. camara* and *H. mutabilis* were not directly affected by insecticides. Insects from known *MED* and *MEAM1* species reference individuals were included in the experiments for comparison with the Tunisia samples. These insect was obtained from the IACR, Rothamsted, UK.

2.2. Determination of *B. tabaci* species

Genomic DNA was extracted from each individual adult of *B. tabaci* in 26 mL of Nonidet P–39 extraction buffer [25] and stored at –20°C. The determination of *B. tabaci* species was based on fixed differences of the mitochondrial cytochrome oxidase I (mtCOI) sequences between species [23]. It was performed through two molecular tests developed previously to enable a rapid and convenient method to discriminate among species: (a) the amplification of species–specific 816 bp mtCOI fragments [23], and (b) the enzymatic digestion of the mtCOI PCR amplified fragment by endonucleases (PCR–RFLP), namely, *VspI* [24], *TaqI* [21] and *AluI* [22] in our study.

For the 146 populations, 10 to 20 insects were analyzed from each population, giving a total of 2099 samples. In each assay, together with the open field and greenhouse collected whiteflies, two individuals from known *MEAM1 and MED* species were included as reference. PCR reaction was performed in a final volume of 25 µl containing 100 ng of each primer [23] 5 µl of 10X buffer, 1 µl of dNTP (10 mM each), 1.5 µl MgCl2 (1.5 mM), 1 U of *Taq* DNA polymerase (Roche, Paris France), 2 µl of template extracted DNA, and sterile water. The amplification was carried out in a PCR Express Thermal Cycler (Bio–Rad) with the following steps: 5 min. at 94 °C (one cycle), followed by 35 cycles of 1 min. at 94 °C, 1 min. at 54 °C and 1 min. at 72 °C and a final extension step of 10 min. at 72 °C. The presence of mt COI amplicons was visualized by electrophoresis in 1.5% agarose gel stained with ethidium bromide.

The PCR products were then digested by the restriction endonucleases (PCR–RFLP), namely, *VspI* (Fermentas) [24], *TaqI* (Fermentas) [21] and *AluI* (Fermentas) [22] which generates clear polymorphism between *MEAM1* and *MED* species. To compare populations collected in Tunisia, two individuals from known *MED and MEAM1* species were included in the test. A 1 kb and 100 bp DNA ladder was used as a marker to determine fragment sizes. The digested DNA was visualized by electrophoresis in 2% agarose gel stained with ethidium bromide.

2.3. Statistical analyses
Comparisons of *B. tabaci* species frequency among host plants types or species (i.e., vegetables vs ornamentals), region and treated/non–treated population were performed by the $\chi 2$ test and the non-parametric Kruskal-Wallis test. All statistical analyses were run with STATGRAPHICS Plus (Manugistics Inc., 1995).

3. Results
3.1. *B. tabaci* species prevalence
A total of 146 populations (2099 individuals) covering an entire region of the *B. tabaci* habitat in Tunisia were surveyed from 2009 to 2013 (Fig. 1, Table 1). The species diversity in each population was tested using polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP) (*TaqI, VspI* and *AluI*) of the mitochondrial cytochrom oxidase I (mtCOI) gene fragment. The control restriction endonuclease digestions with *TaqI*, *VspI* and *AluI* yielded bands of expected size for the reference insects of known *MEAM1* or *MED* species. The three PCR–RFLP assays of all Tunisian individuals of *B. tataci* comply with the given restriction patterns for the *MEAM1* or *MED* species. The digestion with *TaqI* yielded three bands of approximately 414 bp, 162 bp and 144 bp for *MEAM1* species and two bands of approximately 633 bp and 144 bp for *MED* species; the one with *VspI* produced one band of 800 bp for *MEAM1* species and two bands, one of around 300 bp and one of 500 bp for *MED* species (Fig. 2). The digestion with *AluI* yielded the three major bands (307 bp, 229 bp and 204 pb) among the five (with bands of 124 bp and 15 bp) characterizing the *MED* Q1 species and two major bands (550 bp, 204 bp and 124 bp) among the five (with bands of ≈50 bp and 15 bp) characterizing the *MED* Q2 species.

The PCR-RFLP assays of the 2099 individuals studied were in agreement in giving restriction patterns of *MEAM1* for 1427 females (70.1% of all tested individuals) and *Med* for 627 females (29.9% of all tested individuals) (Table 1). We observed that *MEAM1* and *MED* species are widely distributed across Tunisia. However, the *MEAM1* species was much more prevalent than the *MED* species in the majority of the tested populations. Among the 146 collected populations, 16 were a mixed populations that contained *MEAM1* and *MED* species; 96 contained 100% *MEAM1* species, while only 34 populations contained 100% *MED* species (Table 1). No other *B. tabaci* species were found. As the samples were always taken from the same host plants in all six regions, the plants sampled and the locality were independent. This made it impossible to carry out a global statistical test to find out whether the host plant influences the distribution of the biotypes

3.2. Patterns of host plant species distribution of individuals of *MEAM1* and *Med* species
The abundance of individuals belonging to *MEAM1* species compared to those belonging to *Med* species (assessed from the distribution of the 2099 unambiguously assigned individuals) was found to differ significantly when *B. tabaci* populations were grouped by host plants, (vegetables *vs* ornamentals). Indeed, a difference in the frequency of *MEAM1* and *MED* species was observed between individuals collected on vegetables cultivated crops (potato, tomato, pepper, aubergine, bean, melon, courgette and cucumber) (n=1684) and those collected on the two ornamentals plants (*L. camara and H. mutabilis*) (n=415). Whiteflies collected on vegetable crops were almost entirely represented by *MEAM1* species (86.7%) under protected conditions (greenhouses) and in open fields, with *MED* species representing the remaining 13.3%. On ornamental plants the frequency of *MEAM1* species was very low, only 2.7%. In contrast, whatever the region and the collection date, except for five populations/29, only *MED* specimens were found on the two ornamentals crops *L. camara and H. mutabilis* with a frequency about 97.3% (Table 3). The difference in distribution of *MEAM1* and *MED* species on the two types of host plants was highly significant (χ^2_1 = 1120.3, *1 df*, *P*<0.0001, with Yates' correction) and did not follow a random distribution.

The percentage of *MEAM1* species ranged between 0% and 100% among the 117 populations collected on vegetable crops hosts and between 0% and 20% among the 29 populations collected from the two ornamentals host plants, and was significantly higher on vegetables crops than on ornamental hosts (Kruskal–Wallis test, P = 1). Within cultivated vegetable crops, the proportion of *MEAM1* and *MED* species did not differ significantly between host plant families (*S*olanaceous *vs* *C*ucurbitaceous) (χ^2_1 = 3.1, *1 df*, *P*=0.07, with Yates' correction). In contrast, within each of the two groups of host plants, the proportion of *MEAM1* and *MED* species differ significantly between host plant species (among solanaceous hosts, χ^2_1 = 15.3, *3 df*, *P*<0.001, with Yates' correction; among *C*ucurbitaceous hosts, χ^2_1 = 314.7, *2 df*, *P*<0.001, with Yates' correction).

3.3. Patterns of geographical distribution and evolution over time of individuals of *MEAM1* and *Med* species
In our survey (Table 1, Fig. 1), the two species were found together in the six crop producing regions collected from northern, central and southern Tunisia whatever the collection date, but *MEAM1* species were more frequent in each region (71.9% *MEAM1* North *vs* 28.1% Med North _ 66% *MEAM1* central *vs* 34% *Med* central _ 72.1% MEAM1 South *vs* 27.9% *Med* South) (Table 2). Their distribution at regional scale was characterized by a high percentage of individuals belonging to either the *MEAM1* or the *MED* species, except in 16 populations out of the 146 studied (Table 1), indicating very rare spatial mixing between the two species at local scale. When samples were grouped either by geographical origin or by year of collection, the proportion of *MEAM1* and *MED* species did not differ significantly (between the six region χ^2_1 = 9.7, *5 df*, *P*=0.08, with Yates' correction).

3.4. Proportion of the *MEAM1* and *Med* species between treated/ untreated populations
A difference in the proportion of *MEAM1* and *MED* individuals was observed between the individuals collected on insecticide treated plants (n = 1159) and the individuals collected on untreated plants (n = 785) (Table 1). *MEAM1* individuals largely dominated on treated host plants (83.6%) while *MED* slightly prevailed on untreated host plants (55.1%) (χ^2_1 = 321.1, *1 df*, *P*<0.0001, with Yates' correction). The frequency of *MEAM1* individuals ranged between 40% and 100% among the populations collected on treated host plants and between 0% and 100% among the populations collected on untreated plants and was significantly higher on treated than on untreated plants (Kruskal-Wallis test, *P* = 0.42).

3.5. Proportion of the Q1 and Q2 mitochondrial variants
Among the 627 analyzed whiteflies identified as members of the *MED* species, only two different restriction profiles were obtained by *AluI* showing the occurrence of both Q1 (Western Mediterranean) and Q2 (Eastern Mediterranean) mitochondrial types. In general, Q1 individuals were the most abundant (85% of all sampled individuals). Seven out of 50 populations were

a mixture of both mitochondrial variants, the remaining 43 populations represented entirely by Q1 (38 populations) or Q2 (5 populations collected from ornamentals plants) (data not shown). Importantly, the Q2 mitochondrial variant was never detected on cultivated vegetable crops at all periods of the sampling survey.

Fig 1: Map of Tunisia showing the six vegetable producing regions where the 146 *Bemisia tabaci* populations were sampled.

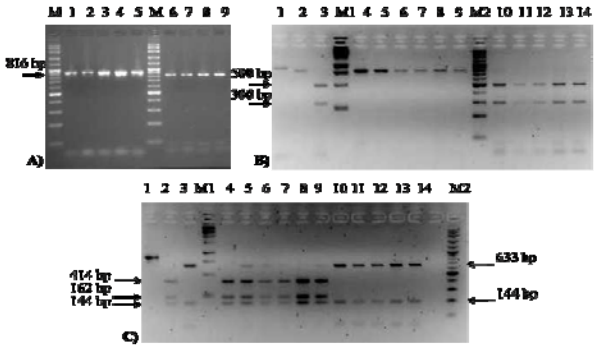

Fig 2: A) PCR amplification of mtCOI (~816bp) from *B. tabaci* popultion collected in korba from Melon. M: DNA marker 100 bp DNA Ladder (Invitrogen): 1 *MEAM1* species control, 2 *MED* species control, 3-9 samples collected from Melon (Mars 2009). B) RFLP patterns of mtCOI amplicons cleaved with *Vsp*I, 1 undigested DNA, 2 *MEAM1* species controls, 3 *MED* species control, M1: DNA marker 1Kb DNA Ladder (Invitrogen), 4-9 samples collected from Melon (Mars 2009), M: DNA marker 100 bp DNA Ladder (Invitrogen), 10-14 samples collected from Lantana (November 2009). C) RFLP patterns of mtCOI amplicons cleaved with *Taq*I, 1 undigested DNA, 2 *MEAM1* species controls,3 *MED* species control, M1: DNA marker 1Kb DNA Plus Ladder (Invitrogen), 4-9 samples collected from Melon (Mars 2009), M: DNA marker 100 bp DNA Ladder (Invitrogen), 10-14 samples collected from Lantana (November 2009).

Table 1: Collection of *B. tabaci* populations in Tunisia from 2009 to 2013 and proportion of each species according to the 146 populations.

Geographic origin	Host plant		Control	Collection data	Total individuals	Number of species		Percentage of *MEAM1*/*MED* (%)
Localities	Name	Collection sites				*MEAM1*	*MED*	
Korba	Melon	Greenhouse	Pyrs and OPs	Mar-2009	15	15	0	100/0
	Courgette	Open field	untreated	Mar-2009	15	15	0	100/0
	Pepper	Open field	Pyrs and OPs	May-2009	15	15	0	100/0
	Cucumber	Greenhouse	neonicotinoid	May-2009	15	0	15	0/100
	Tomato	Greenhouse	Pyrs and OPs	Nov-2009	15	15	0	100/0
	Aubergine	Open field	untreated	Nov-2009	15	15	0	100/0
	Lantana	Public garden	untreated	Nov-2009	15	0	15	0/100
	H. mutabilis	Public garden	untreated	Nov-2009	15	0	15	0/100
	Bean	Open field	untreated	Oct-2009	15	15	0	100/0
	Potato	Open field	Pyrs and OPs	Dec-2009	15	15	0	100/0
	All host				150	105	45	70/30
	Lantana	Near Greenhouse	untreated	Mar-2010	10	0	10	0/100
	Melon	Greenhouse	Pyrs and OPs	Mar-2010	20	20	0	100/0
	Courgette	Open field	-	Mar-2010	20	20	0	100/0
	Tomato	Open field	Pyrs and OPs	Sep-2010	15	15	0	100/0
	Lantana	Public garden	untreated	Sep-2010	10	0	10	0/100
	H. mutabilis	Public garden	untreated	Sep-2010	15	0	15	0/100
	Pepper	Greenhouse	Pyrs and OPs	Oct-2010	20	20	0	100/0
	Aubergine	Open field	untreated	Nov-2010	10	4	6	20/80
	Potato	Open field	Pyrs and OPs	Dec-2010	20	20	0	100/0
	All host				140	99	41	70.7/29.3
	Tomato	Open field	Pyrs and OPs	Jan-2013	15	15	0	100/0
	Courgette	Open field	-	Mar-2013	20	20	0	100/0
	Lantana	Near greenhouse	untreated	Apr-2013	15	2	13	13.4/86.6
	Pepper	Greenhouse	Pyrs and OPs	Apr-2013	20	18	2	90/10
	Melon	Open field	-	Apr-2013	15	15	0	100/0
	Potato	Open field	neonicotinoid	May-2013	20	0	20	0/100
	Cucumber	Greenhouse	Pyrs and OPs	May-2013	15	15	0	100/0
	Bean	Open field	untreated	Nov-2013	15	15	0	100/0
	Aubergine	Open field	Pyrs and OPs	Nov-2013	20	20	0	100/0
	H. mutabilis	Public garden	untreated	Dec-2013	15	0	15	0/100
	All host				170	120	50	70.5/29.5

Bizerte	Pepper	Greenhouse	Pyrs and OPs	Jan-2009	15	15	0	100/0
	Melon	Greenhouse	neonicotinoid	Mar-2009	15	0	15	0/100
	Cucumber	Greenhouse	Pyrs and OPs	Mar-2009	15	15	0	100/0
	Courgette	Open field	untreated	Apr- 2009	10	10	0	100/0
	Aubergine	Open field	untreated	Nov- 2009	10	10	0	100/0
	Bean	Open field	untreated	Dec-2009	15	15	0	100/0
	Potato	Open field	Pyrs and OPs	Dec-2009	10	2	8	20/80
	All host				90	67	23	74.5/25.5
	Pepper	Greenhouse	Pyrs and OPs	Jan-2010	10	10	0	100/0
	Tomato	Greenhouse	Pyrs and OPs	Feb-2010	10	10	0	100/0
	Courgette	Open field	untreated	Mar-2010	10	0	10	0/100
	Lantana	Public garden	untreated	Apr-2010	15	0	15	100/0
	Melon	Greenhouse	Pyrs and OPs	May-2010	15	15	0	0/100
	Pepper	Open field	Pyrs and OPs	Jul-2010	10	10	0	100/0
	Tomato	Open field	untreated	Oct-2010	10	7	3	70/30
	Aubergine	Open field	-	Nov- 010	15	15	0	100/0
	Potato	Greenhouse	Pyrs and OPs	Dec-2010	20	20	0	100/0
	All host				115	87	28	75.7/24.3
	Tomato	Open field	Pyrs and OPs	Mar- 2013	10	10	0	100/0
	Courgette	Open field	untreated	Mar-2013	15	15	0	100/0
	Melon	Greenhouse	neonicotinoid	Apr- 2013	15	0	15	0/100
	Cucumber	Greenhouse	Pyrs and OPs	Apr-2013	15	15	0	100/0
	Potato	Open field	-	Nov-2013	15	15	0	100/0
	Aubergine	Open field	-	Nov-2013	15	15	0	100/0
	Lantana	Near greenhouse	untreated	Dec-2013	15	0	15	0/100
	Pepper	Greenhouse	Pyrs and OPs	Dec-2013	15	13	2	84.7/15.3
	All host				115	83	32	72.1/27.9
Monastir	Melon	Greenhouse	Pyrs and OPs	Mar-2009	15	15	0	100/0
	Cucumber	Greenhouse	Pyrs and OPs	Mar-2009	15	15	0	100/0
	Courgette	Greenhouse	Pyrs and OPs	Apr-2009	20	20	0	100/0
	Tomato	Greenhouse	neonicotinoid	May-2009	15	5	10	33.4/66.6
	H. mutabilis	Near greenhouse	untreated	Nov-2009	15	0	15	0/100
	Lantana	Near greenhouse	untreated	Nov-2009	15	2	13	15.3/84.7
	Potato	Open field	Pyrs and OPs	Dec- 2009	15	15	0	100/0
	All host				110	72	38	66.5/34.5
	Potato	Greenhouse	Pyrs and OPs	Mar-2010	15	15	0	100/0
	Melon	Greenhouse	neonicotinoid	Mar-2010	15	0	15	0/100
	Cucumber	Greenhouse	Pyrs and OPS	May-2010	15	15	0	100/0
	Lantana	Near greenhouse	untreated	Nov-2010	15	0	15	0/100
	Courgette	Open field	untreated	Oct-2010	15	15	0	100/0
	Tomato	Open field	untreated	Oct-2010	15	15	0	100/0
	Pepper	Open field	-	Nov-2010	15	10	5	66.7/33.3
	Aubergine	Open field	Pyrs and OPs	Nov-2010	15	15	0	100/0
	H. mutabilis	Public garden	untreated	Dec-2010	10	0	10	0/100
	All host				130	85	45	65.3/34.7
	Pepper	Greenhouse	Pyrs and OPs	Feb-2013	15	15	0	100/0
	Lantana	Near greenhouse	untreated	Feb-2013	10	3	7	30/70
	Potato	Open field	untreated	Feb-2013	15	15	0	100/0
	H. mutabilis	Public garden	untreated	Apr-2013	15	0	15	0/100
	Cucumber	Greenhouse	neonicotinoid	Jul-2013	15	0	15	0/100
	Tomato	Greenhouse	Pyrs and OPs	Jul-2013	15	15	0	100/0
	Aubergine	Open field	untreated	Nov-2013	15	15	0	100/0
	Lantana	Public garden	untreated	Nov-2013	10	0	10	0/100
	Bean	Open field	untreated	Dec-2013	15	15	0	100/0
	Courgette	Open field	untreated	Dec-2013	15	15	0	100/0
	All host				140	93	47	66.4/33.6
Kairouan	Melon	Greenhouse	Pyrs and OPs	Apr-2009	15	15	0	100/0
	Cucumber	Greenhouse	Pyrs and OPs	May-2009	14	14	0	100/0
	Tomato	Open field	Pyrs and OPs	May-2009	15	15	0	100/0
	Pepper	Greenhouse	neonicotinoid	May-2009	15	0	15	0/100
	Aubergine	Open field	Pyrs and OPs	Oct-2009	15	15	0	100/0
	H.	Public garden	untreated	Nov-2009	15	0	15	0/100

	mutabilis							
	All host				89	59	30	66.2/33.8
	Potato	Open field	Pyrs and OPs	Jan-2010	10	10	0	100/0
	Melon	Greenhouse	neonicotinoid	May-2010	10	10	0	100/0
	Cucumber	Greenhouse	neonicotinoid	Jul-2010	10	10	0	100/0
	Lantana	Near greenhouse	untreated	Jul-2010	15	0	15	0/100
	Tomato	Greenhouse	-	Nov-2010	15	15	0	100/0
	Lantana	Near greenhouse	untreated	Nov-2010	15	1	14	6.7/93.3
	Potato	Open field	-	Dec-2010	10	10	0	100/0
	All host				85	56	29	65.8/34.2
	Potato	Open field	Pyrs and OPs	Jan- 2013	15	0	15	0/100
	Courgette	Open field	Pyrs and OPs	Mar-2013	10	2	8	20/80
	Melon	Open field	Pyrs and OPs	May-2013	15	15	0	100/0
	Cucumber	Open field	untreated	May-2013	15	15	0	100/0
	Tomato	Open field	untreated	Sep- 2013	15	15	0	100/0
	Pepper	Open field	Pyrs and OPs	Oct-2013	15	15	0	100/0
	Aubergine	Open field	Pyrs and OPs	Dec-2013	15	15	0	100/0
	Lantana	Public garden	untreated	Dec-2013	15	0	15	0/100
	All host				115	77	38	66.9/33.1
Gabes	Potato	Greenhouse	Pyrs and OPs	Jan-2009	15	15	0	100/0
	Courgette	Open field	untreated	Mar-2009	15	15	0	100/0
	Lantana	Near greenhouse	untreated	Apr- 2009	20	0	20	0/100
	Cucumber	Greenhouse	Pyrs and OPs	Apr-2009	15	15	0	100/0
	Pepper	Greenhouse	Pyrs and OPs	May-2009	15	15	0	100/0
	Aubergine	Open field	untreated	Sep-2009	15	15	0	100/0
	Tomato	Greenhouse	Pyrs and OPs	Nov-2009	10	10	0	100/0
	Lantana	Public garden	untreated	Dec-2009	20	0	20	0/100
	All host				125	85	40	68/32
	Tomato	Greenhouse	neonicotinoid	Feb-2010	10	0	10	0/100
	Lantana	Near greenhouse	untreated	Feb-2010	15	0	15	0/100
	Courgette	Greenhouse	Pyrs and OPs	Mar-2010	15	15	0	100/0
	Melon	Greenhouse	Pyrs and OPs	Apr-2010	15	15	0	100/0
	Aubergine	Open field	untreated	Oct-2010	15	15	0	100/0
	Potato	Greenhouse	Pyrs and OPs	Dec- 2010	15	15	0	100/0
	All host				85	60	25	70.5/29.5
	Tomato	Greenhouse	Pyrs and OPs	Feb-2013	15	15	0	100/0
	Pepper	Greenhouse	Pyrs and OPs	Mar-2013	15	15	0	100/0
	Melon	Greenhouse	Pyrs and OPs	Mar-2013	10	10	0	100/0
	Lantana	Near greenhouse	untreated	Mar-2013	15	0	15	0/100
	Aubergine	Open field	Pyrs and OPs	Sep-2013	20	20	0	100/0
	Potato	Open field	-	Nov-2013	15	15	0	0/100
	Lantana	Public garden	untreated	Nov-2013	15	0	15	0/100
	All host				105	75	30	71.4/28.6
Kebili	Potato	Open field	Pyrs and OPs	Jan-2009	15	15	0	100/0
	Melon	Greenhouse	neonicotinoid	Mar- 2009	10	0	10	0/100
	Cucumber	Greenhouse	Pyrs and OPs	Mar-2009	15	15	0	100/0
	Tomato	Greenhouse	Pyrs and OPs	Apr-2009	15	15	0	100/0
	Pepper	Greenhouse	neonicotinoid	Apr-2009	15	6	9	40/60
	Melon	Open field	untreated	Jul-2009	20	20	0	100/0
	Aubergine	Greenhouse	Pyrs and OPs	Sep-2009	15	15	0	100/0
	H. mutabilis	Near greenhouse	untreated	Sep-2009	15	3	12	20/80
	All host				120	89	31	74.1/25.9
	Melon	Greenhouse	Pyrs and OPs	Mar-2010	15	15	0	100/0
	Cucumber	Open field	untreated	Apr-2010	15	15	0	100/0
	Courgette	Greenhouse	Pyrs and OPs	Apr-2010	15	15	0	100/0
	Tomato	Greenhouse	Pyrs and OPs	May-2010	15	15	0	100/0
	Pepper	Greenhouse	neonicotinoid	May-2010	15	11	4	26.7/73.3
	Lantana	Near greenhouse	untreated	May-2010	15	0	15	0/100
	Aubergine	Open field	untreated	Nov- 2010	15	7	8	46.7/53.3
	All host				105	78	27	74.2/25.8
	Cucumber	Greenhouse	Pyrs and OPs	Apr-2013	15	15	0	100/0
	Melon	Greenhouse	Pyrs and OPs	May- 2013	15	15	0	100/0
	Lantana	Near	untreated	May- 2013	10	0	10	0/100

	greenhouse						
Courgette	Open field	Pyrs and OPs	May 2013	10	10	0	100/0
Tomato	Open field	untreated	Nov 2013	15	14	1	6.7/93.3
Potato	Open field	Pyrs and OPs	Nov-2013	15	15	0	100/0
Aubergine	Open field	Pyrs and OPs	Nov-2013	15	13	2	13/83
Lantana	Near greenhouse	untreated	Nov-2013	15	0	15	0/100
All host				110	82	28	74.5/25.5

Table 2: Species prevalence (%) of the Tunisian samples according to the geographical origin and collection data.

Geographic origin	Collection data	Total individuals	Number of species		Percentage of *MEAM1*/*MED* (%)
			MEAM1	*MED*	
Korba	2009	150	105	45	70/30
	2010	140	99	41	70.7/29.3
	2013	170	120	50	70.5/29.5
		460	324	136	70.4/29.6
Bizerte	2009	90	67	23	74.5/25.5
	2010	115	87	28	75.7/24.3
	2013	115	83	32	72.1/27.9
		320	237	83	74.1/25.9
North		780	561	219	71.9/28.1
Monastir	2009	110	72	38	66.5/34.5
	2010	130	85	45	65.3/34.7
	2013	140	93	47	66.4/33.6
		380	250	130	65.7/34.3
Kairouan	2009	89	59	30	66.2/33.8
	2010	85	56	29	65.8/34.2
	2013	115	77	38	66.9/33.1
		289	192	97	66.4/33.6
Central		669	442	227	66/34
Gabes	2009	125	85	40	68/32
	2010	85	60	25	70.5/29.5
	2013	105	75	30	71.4/28.6
		315	220	95	69.8/30.2
kebili	2009	120	89	31	74.1/25.9
	2010	105	78	27	74.2/25.8
	2013	110	82	28	74.5/25.5
		335	249	86	74.3/25.7
South		650	469	181	72.1/27.9
Total		2099	1472	627	70.1/29.9

Table 3: Species prevalence (%) of the Tunisian populations according to host plant sampled.

Host-plant	Total individuals	*MEAM1* species	*MED* species	Percentage of *MEAMI /MED* (%)
H. mutabilis	115	3	112	2.7/97.3
Lantana	300	8	292	2.7/97.3
All ornamentals plants	415	11	404	2.7/97.3
Tomato	245	221	24	90.2/9.8
Pepper	225	188	37	83.5/16.5
Potato	255	212	43	83.1/16.9
Aubergine	240	224	16	93.3/6.7
All *Solanaceous* plants	965	845	120	87.5/12.5
Melon	250	195	55	78/22
Cucumber	204	174	30	85.2/14.8
Courgette	205	187	18	91.2/8.8
All Cucurbits plants	659	556	103	84.3/15.7
bean	60	60	0	100/0
All vegetable crops	1684	1461	223	86.7/13.3
Total	2099	1472	627	70.1/29.9

4. Discussion

An extensive survey of *B. tabaci* populations covering the northern (Bizerte and Korba), central (Monastir and Kairouan) and southern (Gabes and Kibili) of Tunisia was conducted from 2009 until 2013 in order to determine the distribution of species of the *B. tabaci* complex. Our results indicated the presence of only two species in Tunisia: the *Middle East-Asia Minor1* and the *Mediterranean*. These species were found together in each region during all periods of the sampling survey. However, we observed a large spreading and dominance of *MEAM1* species (70.1% of all sampled individuals) on *MED* species whatever the region and the date of collection. We compared our results with prior research papers, especially Saleh *et al.* [17], and confirmed that there are a considerable change in the relative abundance of *MEAM1* and *MED* species. Indeed, from 2006 to 2008, 54.5% of the *B. tabaci* collected belonged to the *MEAM1* species, while the remaining populations belonged to the *MED* species. The *MEAM1* species were significantly more frequent in the north and *MED* species in the south. Moreover, the percentage of *MEAM1* species among the populations collected on tomato, aubergine and courgette was 80%, 61.5% and 82.1% respectively from 2006 to 2008 and 90.2%, 93.3% and 91.2% respectively from 2009 to 2013.

In Tunisia, in contrast to usual observations in the Mediterranean basin, *MEAM1* and *MED* species have been

seen to co-occur in the all regions, but some differences in the species dynamics were recorded according to host plants. *MEAM1* and *MED* species were clearly seen to co-occur at the regional scale at all periods of the sampling survey, but to mutually exclude one another at the sample scale. *MEAM1* species resulted largely predominant (86.7%) on vegetable crops under protected conditions and in open fields, with *MED* species representing the remaining 13.3%, while *MED* species was consistently found on ornamental plants (97.3%) cultivated near vegetables crop production and in public gardens. Our results provide additional evidence for the occurrence of host associations between *MEAM1* or *MED* species and host type (vegetable/ornamental) in Tunisia. The extent to which *B. tabaci* populations restrict themselves to specific hosts in space and time will likely influence the population dynamics related to the development and spread of traits such as insecticide resistance in the same area. The dominance of *MEAM1* species on cultivated plants, leads to the conclusion that this species might be the predominant resident begomovirus vector group on market gardening crops. However, although *MEAM1* species was found to be predominant on vegetable crops, it appeared not able to persist and compete with *MED* species on ornamental plants. Currently, *MED* and *MEAM1* species are the most frequently observed species in the Mediterranean basin, they are often observed separately [15, 19, 20]. Where multiple species of the *B. tabaci* complex-above all *MED* and *MEAM1* have co-occurred for some time, their distributions have often been reported to be highly dynamic in space and time. After a period of co-existence, only one species is detected in field surveys, because under specific conditions evaluated in the laboratory and using models, one species may be favoured over another [9, 10, 11, 13, 26]. Consequently, in some regions, *MED* colonization has resulted in the displacement of the *MEAM1*, particularly where insecticides are applied frequently, due to the higher insecticide resistance, higher fitness of resistant *MED* populations [11] and greater tolerance to high temperatures [27]. However, *MED* and *MEAM1* exhibit different responses to host plants (species and cultivars) in terms of life-history traits and population dynamics [27, 28]. Therefore, each species can have an advantage over the other depending on the specific environmental and agricultural context. Thus, in recent years, in Mediterranean countries such as Italy [29], Spain [30] and Morocco [20], *MEAM1* species have been reported to have been supplanted or displaced by *MED B. tabaci* species. The displacement of an earlier invasive *B. tabaci* race by a new invasive race has been reported in several countries such as Australia [7] and China [26]. In Tunisia, the distribution patterns of *MEAM1* and *MED B. tabaci* species were so distinct that it obviously cannot be due to chance. Our results clearly showed that, in the Tunisian agricultural system, each species has a different habitat. The factors that are driving this distribution in Tunisia are not completely evident from the present work, and future research needs to be carried out. However, based on our results some hypotheses can be drawn.

We found a different proportion of *MEAM1* and *MED* species on different host plants. *MEAM1* species resulted largely predominant (86.7%) on vegetables hosts, while *MED* species dominates (97.3%) on ornamentals plants. This result suggests that the fitness of *MEAM1* species on vegetables hosts could be higher than *MED* species. Plants of the *Solanaceae* and *Cucurbitaceae* families are very common in the six producing regions. Field and greenhouse crops, including potato, tomato, pepper, aubergine, bean, melon, courgette and cucumber are largely cultivated throughout the year, providing the opportunity of a continuous reproduction to the insects. Selection pressure induced by host-plant may be is a crucial factor promoting this distribution. Indeed, some *B. tabaci* populations are known to have a restricted host-plant range [7] but, to the best of our knowledge, host-plant specialization or host plant preference has never been reported in the highly polyphagous *MEAM1* and *MED* species. Selection pressures imposed by each plant species is driving diversification despite the overlapping distribution of host plant species. The evolution of specific host races is thought to represent the incipient stage of sympatric speciation which is perhaps best exemplified through the apple maggot fly, *Rhagoletis pomonella* Walsh (Diptera: Tephritidae) [31]. In the case of *B. tabaci*, there is considerable discussion relating to the apparent variation across the complex in regards to capacity to utilize different host plant species. However, our knowledge of host range across the *B. tabaci* complex is decidedly patchy with much of our knowledge being assumed on the basis of scant comparisons against the highly invasive *MED* and *MEAM1* both of which have a very broad host range. As such, the hypothesis posed by [8], that variation in host plant utilization was driving diversity within *B. tabaci*, has yet to be adequately tested. In Tunisia specialization, if it exists, is probably still in progress or is too recent to be genetically significant among the different populations.

Another reason for this distribution could be the agricultural practices such as insecticide treatments. Insecticides may play crucial role in affecting the distribution and frequency of whitefly species across regions [13]. In Tunisia, chemical treatments are the main method of control of *B. tabaci* and several other pests such as Aphids. Although other chemical classes have also recently been used against whiteflies, organophosphates and pyrethroids have long been the most widely applied chemistries, along with neonicotinoids. Insecticide resistance has often been assumed to play a major role in the distribution of *B. tabaci* in agricultural systems. Thus, the *MED* species is reported to be prevalent over *MEAM1* species in agricultural environments, and this is usually explained by the reduced susceptibility of *MED* species to insecticides [11, 13]. This has been documented in many countries worldwide, including several Mediterranean countries [11]. However, our study confirmed that *MEAM1* was prevalent over *MED* on vegetable crops under greenhouse and in open field, and was rarely found at all on the ornamental outside the context of cropping systems. Further, we found a higher proportion of *MEAM1* species on insecticide treated (83.6%) than on untreated host plants (44.9%), suggesting that insecticide resistance and fitness of resistant individuals of *MEAM1* species might be higher than *MED* species. Because in the six vegetables producing regions insecticides are currently applied on vegetable to control *B. tabaci* and other pests and not to ornamental crops, selection pressure induced by insecticides may be a crucial factor promoting the predominance of *MEAM1* species on vegetable crops. The most susceptible *B. tabaci* were found in environments that receive fewer insecticide treatments, the most susceptible species would logically be *MED* populations on the ornamentals crops, clearly contradicting observations usually reported in the literature. Consequently, if differential resistance to insecticides interferes in the distribution of *B. tabaci* in Tunisia, it may be in a different way from that usually described. Recently, Gauthier *et al.* [32] showed that Tunisian *MED* populations displayed very low frequencies of resistance alleles for *Kdr* and *Ace* genes. As a result, resistant mutations were almost absent in most regions of Tunisia, except in El Hamma (Gabes). However, to date, nothing is known about the Tunisian *MEAM1* species resistance to

insecticides. Instead, this host-plant distribution, which probably results from competition between *MEAM1* and *MED* [17,33] would enable *MED B. tabaci* to take refuge in an insecticide-free space and/or prevent *MED B. tabaci* from colonising a more suitable resource for development, such as vegetable crops, where resistance mutations would have the opportunity to be selected and increase in frequency. The absence of target-site resistance in *MED* Tunisian populations does not necessarily mean that there is no resistance to insecticides, as other mechanisms, not detectable by the present diagnostic assays, may be at the origin of resistant phenotypes. For instance, carboxy esterases and P440 monooxygenases have been associated with metabolic resistance of *B. tabaci* against a number of OPs and Pyrs [34]. Such agro-ecological context may explain the predominance of *MEAM1* species populations that has occurred in Tunisia.

Finally, this distribution of the two species of *B. tabaci* in Tunisia could result from the infestation of its symbionts. The whitefly *B. tabaci* harbors the primary symbiont, *Portiera aleyrodidarum* and a number of secondary symbionts including: *Hamiltonella, Arsenophonus, Wolbachia, Fritschea*, *Cardinium*, and *Rickettsia.* These symbionts affect many aspects of the insect's biology but many of these aspects are still unknown. *Rickettsia* sp. was first detected in *B. tabaci* in Israel in 2006 in both *MEAM1* and *MED* species [35]. Looking for the infectious status of *B. tabaci* populations by secondary symbionts in Tunisia, Gorsane *et al* [16] suggests a potential correlation between secondary symbionts and *B. tabaci* species. *Hamiltonella, Rickettsia, Cardinium, Wolbachia,* and *Fritschea* were carried among all populations, whereas *Arsenophonus* was not detected in any tested individuals. *Hamiltonella* and *Cardinium* are the only endosymbionts shared by both species. *Wolbachia* and *Fritschea* were detected in *MED* species whereas *Rickettsia* was present in *MEAM1* species only. An interesting study on the relationship between *B. tabaci* and *Rickettsia* sp. was conducted in the SW USA [36]; they concluded that since 2000 this symbiont has expanded its infestation in the host, *B. tabaci MEAM1* species, and this infestation increased the fitness of the whitefly. These results contrast the findings from Israel where no reproductive manipulation associated with *Rickettsia* infection in the *MEAM1* species of *B. tabaci*. The reason for this dissimilarity is unclear, suggesting that there is difference between the Israeli and US *B. tabaci- Rickettsia* interaction [36]. In the case of *Wolbachia*, the 30.76% infection rate reported in Tunisia collection was in agreement with results of Chiel *et al.* [35] who reported the presence of *Wolbachia* only *MED* species in Israel with a proportion of 30%. This bacterium had been shown to give some protection against parasitism and increased fitness on an invasive *B. tabaci MED* population in China [37]. On the other hand, a high incidence of *Hamiltonella,* which is harbored by 100% of *MED* species and 90.9% of *MEAM1* species. Such a high incidence was reported only for the Israeli *MEAM1* species (100%) because the *MED* species did not carry this symbiont. *Hamiltonella* was detected in the both *MEAM1* and *MED* species in *B. tabaci* populations collected across Croatia showing the highest prevalence among secondary symbionts [38]. Even the function of these secondary symbionts is yet no elucidated, their association within *B. tabaci* suggests a possible contribution of these bacteria to insect ecology and evolution. Secondary symbionts may be involved in increasing tolerance to heat stress [39] and causing host-plant specialization [40].

5. Conclusion

In this manuscript, the species diversity of the whitefly *B. tabaci* was studied from various plants in Tunisia. We identified the same biotypes as had previously been reported by Saleh *et al* [17]. Our results clearly demonstrate that the *MEAM1* species dominates in vegetable crops while, the *MED* species dominates in ornamental crops. In Tunisia, *MEAM1* and *MED B. tabaci* were clearly seen to co-occur at regional scale at all periods of the sampling survey, but to mutually exclude one another at the sample scale. It is therefore essential to develop Integrated Resistance Management (IRM) programs that take into account this distribution. These programs should be based on continuous resistance monitoring surveys, better technologies in insecticide applications and alternations between insecticides with different mode of actions. The particular task must be involved the determination of the Tunisian *MEAM1* species resistance level against organophosphate, pyrethroid and neonicotinoid insecticides, the major insecticide classes extensively used for pest management in vegetables crops.

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7. Disclosure

No conflict of interests is involved in the publication of this article.

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