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DIVERSITY AND ABUNDANCE OF LANDING APHIDS IN TWO AREAS OF SEED POTATO PRODUCTION IN TUNISIA; RELATIVE TO THE INCIDENCE OF POTATO VIRUSES

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Hlaoui A., Mazzoni E., Souissi R., Boukhris-Bouhachem S. – Diversity and abundance of landing aphids in two areas of seed potato production in Tunisia; relative to the incidence of potato viruses.

Aphid flight activities were monitored in 2015 in two areas of seed potato production in Tunisia. Yellow water traps were used to investigate the diversity of aphidofauna and the incidence of aphids involved in potato virus transmission. A total of 9966 specimens belonged to 73 taxa were captured. The cumulative abundance recorded in Douala (first site) was much higher than in El Oglia (second site) (8768 vs 1189 specimens). The most preponderant species in Douala were *Aphis spiraecola* Patch (66.1%) and *Aphis* spp. group (13.5%). In El Oglia *Rhopalosiphum padi* (L.) comprised 58.9% of total catches, followed by *Acyrtosiphon pisum* (Harris) (12%) and *Aploneura lentisci* (Passerini) (10.4%). The highest values of diversity indices were recorded on April 23th [Shannon-Wiener index (H')=2.58; Simpson index (1-D)=0.88] in Douala and on May 12th (H' =1.70; 1-D=0.75) in El Oglia. The detection of viral infections by DAS-ELISA determined the predominance of *Potato virus Y* amongst five main potato viruses tested. Overall Chi-square analysis determined significant differences crossing all areas of seed and ware potatoes and season crops. The highest incidence of viruses was recorded in Douala (18.5%) while El Oglia was under the threshold value (4%). This might be attributed to the occurrence of aphids in early season known previously to be efficient vectors of PVY and to the characteristics of area including climatic conditions, altitude and landscape structure. These results provide new evidence for El Oglia as ideal site to produce healthy tubers while conversely Douala is favourable for the propagation of vectors and viruses.

KEY WORDS: aphid abundance, diversity indices, species richness, potato virus incidence, PVY, seed potato.

INTRODUCTION

In Tunisia around 46,000 tons of potato seed are planted annually in three cropping seasons: early (from October-January to February-May), main (from January-February to May-June) and late (from August-September to December-January). Around 20,000 tons are imported from Europe as A-class seeds with 5% virus tolerance planted in the main season. 20,000 tons of late potato plants often originate from self-produced seeds by growers with 80% of this quantity and 20% provided by Groupement Interprofessionnel des Légumes (GIL) as Elite-class with 2% virus tolerance. The remaining quantity (6,000 tons), are planted in the early season with 50% from European imported seeds, 30% provided by GIL and 20% by Tunisian growers (BOUKHRIS-BOUHACHEM *et al.*, 2010; GIL, 2014). Since the establishment of national programme for seed multiplication and production, five regions have been involved in seed multiplication and production: Cap Bon (in Nabeul), Manouba, Beja, Jendouba and Kairouan. Cultivar Spunta ranked as the major seed planted throughout the year (more than 70%) in comparison with the other cultivars, owing to its adaptive

characteristics to soil, climatic conditions and mainly due to its resistance to virus dissemination.

GIL was responsible for the production, multiplication and distribution of the certified seeds; preventive measures were required to ensure quality and check that the tubers were healthy and free from viruses (e.g., the use of resistant cultivars, aphid population management, visual checks and post-harvest diagnosis). Despite this control strategy each year, almost 12% of seed potato fields have to be downgraded because rates of virus infection exceed the levels of virus tolerance required by Tunisian legislation (GIL, 2014; KHAMASSY *et al.*, 2015).

In fact, viral diseases are the principal cause of damage in seed potato production. Two types of virus transmission occur: the first (vertical transmission, involving transmission of a virus from mother tuber to daughter) arises from sowing local tubers kept by growers from the previous main season. The second (called horizontal transmission) is caused by aphid vectors during the feeding process, which can cause dramatic yield losses and consequently this generates the problem of unmarketable potatoes (LACOMME *et al.*, 2017).

Although more than 40 viruses affect potatoes (VALKONEN, 2007), only five common viruses are included in the Tunisian certification scheme, *Potato virus Y* (PVY, genus *Potyvirus*), *Potato virus A* (PVA, genus *Potyvirus*), *Potato Virus S* (PVS, genus *Carlavirus*), *Potato leaf roll Virus* (PLRV, genus *Polerovirus*) and *Potato virus X* (PVX, genus *Potexvirus*). These viruses are known to be the most damaging to potato crops worldwide (KERLAN, 2008). Except for PVX, which is transmitted only mechanically, the remaining viruses from the *Potyvirus*, *Polerovirus* and *Carlavirus* genera are transmitted by aphid vectors (RADCLIFFE and RAGSDALE, 2002).

In this light, the interaction between aphid borne viruses and potato plants is of particular concern for seed potato production, because the certification scheme sets specific limits for virus tolerance and aphids tend to play the major role in virus transmission. This is due to their (i) biological features: most commonly winged morphs are able to spread infection over large areas (i.e. winged aphid *Aphis nasturtii* Kaltendach can retain PVY up to 17 h enabling the aphid to spread the virus in other fields) (KOSTIW, 1975); (ii) life cycle: aphids (Hemiptera: Aphididae) exhibit a complex life cycle generally reproducing through cyclical parthenogenesis or obligate parthenogenesis and this type of reproduction promotes heteroecy by increasing aphid populations on the potato and surrounding plants thus enabling aphids to reproduce and to avoid natural enemies, weather pressure and insecticide selection (DIXON, 1998); (iii) physiological and behavioural aspects: aphids insert their stylets and ingest virus from epidermal cells (case of PVY) or reach phloem vessels and acquire virus particles (case of PLRV). However, aphids can transmit viruses in persistent, semi persistent or non persistent ways. The last is the most damaging and worrying because aphids acquire viruses very quickly (within seconds or minutes) and can immediately transmit them to the next plant. Moreover, potato plants infected by PVY affect the landing and feeding behaviour of *Myzus persicae* (Sulzer) by promoting its ingestion and increasing the probing time that increases virus spread (BOQUEL *et al.*, 2011).

Of viruses non-persistently transmitted (around 75%), PVY has the greatest economic impact mainly on potato seed production and about 65 aphid species are implicated in its transmission (LACOMME *et al.*, 2017). Several studies have highlighted the effect of aphid vectors in the epidemiology of PVY as the most destructive virus in seed potato production in Tunisia (CHERIF and BOUDHIR, 1990; CHERIF and MNARI-HATTAB, 1994). In the same context, 50% of yield losses were caused by PVY and PLRV infections, mainly due to planting locally produced seeds which present a risk for pathogen multiplication (CHERIF and BOUDHIR, 1990; KHAMASSY, 1999). In Algeria potato destruction is caused by PVY infections generating 24% of yield losses (ALLALA-MESSAOUDI *et al.*, 2014).

In Egypt the situation is worse, with yield losses at between 50 to 80% (ABDALLA *et al.*, 2016).

Although more than 5000 aphid species have been identified to date, only 100 species are recognised to have an economic impact by weakening crops through direct feeding and virus vectoring (VAN EMDEN and HARRINGTON, 2017). The Aphididae family is responsible for 57% of viruses transmitted (NAULT, 1997). Moreover, Aphids are considered as the predominant vector of viruses in potato production because they are involved in the transmission of 13 potato viruses. Interestingly, aphids that exhibit a high migration capacity are able to find more hosts thus increasing their reproduction capacity and raising the risk of virus incidence (BRUNT, 2001).

The first aim of this study was to track the movement of winged aphids circulating in two fields of seed potato production in Tunisia, in order to assess species richness, aphid diversity (using specific diversity indices) and the risk of vector abundance. For this, two areas of seed potato production were investigated: Douala (the first site) as a reference field from Cap Bon, a traditional region of seed potato production (since the establishment of national program of multiplication and production of seed potatoes in 1977) in comparison with a newly introduced area, El Ogla (the second site) in the Goubellat region.

The trapping system is widely used to follow the fluctuation of aphids, in particular those displaying some potentiality in the transmission of virus diseases. Generally, data provided by the trapping method raise awareness to prevent the risk of virus incidence but this is not sufficient. Therefore, the second aim is to study virus infections at the foliage level, which is essential to predict whether a field is suitable for producing certified potato seeds. To our knowledge, no study in Tunisia has dealt with both aspects (aphid dynamic and virus outbreaks). We undertook diagnosis of the incidence of the five main potato viruses (PVY, PVA, PVS, PLRV and PVX) in three growing areas of seed and ware potatoes in order to compare their occurrence in two cropping seasons (main and late).

MATERIALS AND METHODS

SAMPLING SITES AND TRAPPING METHOD

Aphid flight activities were monitored during the potato growing season, from March to May, 2015 in two areas of seed potato production in Tunisia. The first site was in Douala (36° 50' 34" N, 10° 36' 44" E) in the Cap Bon region, a coastal zone with a sub-humid climate. The second site was located in El Ogla (36° 32' 09" N, 09° 42' 05" E) in the Goubellat region, a continental zone in an upper semi-arid climate (Fig. 1). Winged aphids were collected using yellow water traps (YWTs) (MOERICKE, 1955) placed on the edge of seed potato fields, 0.7 m above soil level. The YWTs were emptied and filtered once a week and the aphids stored

Fig. I - Map of Tunisia showing prospected sites. Black spots correspond to the locations where aphids were surveyed by yellow water traps; white spots correspond to sampling sites of seed and ware potatoes surveyed for the presence of the five main potato viruses.

in 70% ethanol, counted and identified using a stereomicroscope and using keys for aphid identification (JACKY and BOUCHERY, 1988; REMAUDIÈRE and REMAUDIÈRE, 1997; BLACKMAN and EASTOP, 2000).

SEROLOGICAL DETECTION OF FIVE MAIN POTATO VIRUSES

Detection of potato virus infections in seed and ware potatoes was undertaken by serological testing in the three growing areas of Douala, El Ogla and Soliman (Fig. I). Thus, in the main and late seasons potato leaf samples, from cultivar Spunta exhibiting virus symptoms as well as necrosis, mosaic, curling, yellowing and stunting either symptomless were randomly collected from each site following the “Z” or zig-zag shape of each potato field. In total 624 potato leaves were tested by Double Antibody Sandwich Enzyme Linked Immunosorbent Assay (DAS-ELISA) following the protocol described by (CLARK and ADAMS, 1977) using anti bodies for PVY, PVA, PVS, PLRV and PVX from a commercial kit (Bioreba, Basel, Switzerland).

POPULATION DIVERSITY EQUATIONS

Population diversities during the study period were

assessed by several diversity indices calculated using the following formula:

Shannon-Wiener index of diversity (H') = $-\sum_{i=1}^S p_i \ln p_i$; $p_i = \frac{n_i}{N}$ (SHANNON and WEAVER, 1964).

Where p_i is the proportion of individuals in a sample belonging to the total population; n_i is the number of specimens of i^{th} species; N is the total number of specimens; S is the total number of species or species richness.

Pielou's evenness (EH) = $\frac{H'}{\ln S}$ where H' is the Shannon's index of diversity; $\ln S$ is the natural log of species recorded (PIELOU, 1969).

Simpson's diversity index ($1-D$) = $1 - \left(\frac{\sum_{i=1}^S (n_i(n_i-1))}{N(N-1)} \right)$ (SIMPSON, 1949).

Simpson's equitability (Evenness) (ED) = $\frac{1}{\sum_{i=1}^S (p_i^2) (S)}$ (MAGURRAN, 2004).

Table 1 – Aphids captured in yellow water traps during study period of potato growing season, 2015.

Family	Subfamily	Site	Cap Bon	Goubellat
		Region	Douala	El Ocla
		Total specimens	8768	1198
		Total species	59	28
		Total genera	33	21
		Species	%	%
Aphididae	Aphidinae	<i>Acyrtosiphon gossypii</i> Mordvilok	0.01	–
		<i>Acyrtosiphon malvae</i> (Mosley)	0.05	–
		<i>Acyrtosiphon pisum</i> (Harris)	3.51	12.02
		<i>Aphis craccivora</i> Koch	0.17	1.84
		<i>Aphis fabae</i> Scopoli	3.14	0.67
		<i>Aphis fabae cirsiiacantoidis</i> (Scopoli)	0.14	–
		<i>Aphis fabae mordwilko</i> (Börner)	0.06	–
		<i>Aphis fabae solanella</i> Theobald	0.17	–
		<i>Aphis gossypii</i> Glover	1.76	–
		<i>Aphis helianthi</i> (Monell)	0.01	–
		<i>Aphis hypochoeridis</i> Börner	0.01	–
		<i>Aphis idaei</i> van Der Goot	0.01	–
		<i>Aphis nerii</i> Boyer de Fonscolombe	0.02	–
		<i>Aphis origani</i> Passerini	0.01	–
		<i>Aphis pomi</i> De Geer	0.02	–
		<i>Aphis punicae</i> Passerini	0.06	–
		<i>Aphis sambuci</i> L.	0.01	–
		<i>Aphis</i> spp.	13.50	1.09
		<i>Aphis spiraecola</i> Patch	66.07	0.17
		<i>Aulacorthum solani</i> (Kaltenbach)	0.05	–
		<i>Brachycaudus amygdalinus</i> (Schouteden)	0.05	–
		<i>Brachycaudus cardui</i> (L.)	0.14	0.17
		<i>Brachycaudus rumexicolens</i> (Patch)	0.05	–
		<i>Brachycorynella asparagi</i> (Mordvilko)	0.01	–
		<i>Brachyunguis harmalae</i> B. Das	0.06	0.33
		<i>Brevicoryne brassicae</i> (L.)	0.42	–
		<i>Capitophorus elaeagni</i> (del Guercio)	0.05	–
		<i>Cavariella aegopodii</i> (Scopoli)	0.03	–
		<i>Cavariella cicutae</i> (Koch)	0.01	–
		<i>Diuraphis noxia</i> (Kurdjumov)	–	0.08
		<i>Dysaphis apiifolia</i> (Theobald)	0.14	0.33
		<i>Dysaphis foeniculus</i> (Theobald)	0.16	0.58
		<i>Dysaphis pyri</i> (Boyer de Fonscolombe)	0.02	–
		<i>Dysaphis</i> spp.	0.11	0.33
		<i>Dysaphis tulipae</i> (Boyer de Fonscolombe)	0.32	0.33
		<i>Hyadaphis coriandri</i> (B. Das)	0.08	0.25
		<i>Hyadaphis foeniculi</i> (Passerini)	0.05	–
		<i>Hyalopterus amygdali</i> (E. Blanchard)	0.02	–
		<i>Hyalopterus pruni</i> (Geoffroy)	0.02	0.25
		<i>Hyperomyzus carduellinus</i> (Theobald)	0.06	0.08
		<i>Hyperomyzus lactucae</i> (L.)	0.34	1.09
		<i>Hyperomyzus picridis</i> (Börner & Blunk)	0.02	–
		<i>Hyperomyzus</i> sp.	0.01	–
		<i>Lipaphis erysimi</i> (Kaltenbach)	0.29	0.08
		<i>Macrosiphoniella abrotani</i> (Walker)	–	0.17
		<i>Macrosiphum euphorbiae</i> (Thomas)	0.10	0.17
		<i>Macrosiphum rosae</i> (L.)	0.08	0.17
		<i>Melanaphis donacis</i> (Passerini)	0.02	–
		<i>Metopolophium dirhodum</i> (Walker)	1.67	0.08
		<i>Microlophium</i> sp.	0.01	–
		<i>Myzus cerasi</i> (Fabricius)	–	0.08
		<i>Myzus persicae</i> (Sulzer)	0.56	0.42
		<i>Ovatus craetaegarius</i> (Walker)	0.01	–
		<i>Protaphis</i> spp.	0.03	–

	<i>Rhodobium porosum</i> (Sanderson)	0.07	–
	<i>Rhopalosiphum insertum</i> (Walker)	0.01	–
	<i>Rhopalosiphum maidis</i> (Fitch)	–	0.08
	<i>Rhopalosiphum nymphaeae</i> (L.)	0.01	0.08
	<i>Rhopalosiphum padi</i> (L.)	0.59	58.93
	<i>Schizaphis graminum</i> (Rondani)	0.09	0.83
	<i>Sitobion avenae</i> (Fabricius)	0.02	0.08
	<i>Sitobion fragariae</i> (Walker)	0.02	–
	<i>Uroleucon sonchi</i> (L.)	0.01	–
	<i>Uroleucon uromelan</i> sp.	–	0.08
	<i>Uroleucon</i> spp.	0.17	
	<i>Uromelan</i> spp.	0.02	0.08
Pemphiginae	<i>Aploneura lentisci</i> (Passerini)	1.44	10.43
Lachninae	<i>Eulachnus tuberculostemmatus</i> (Theobald)	0.03	–
Eriosomatinae	<i>Geoica lucifuga</i> (Zehntner)	0.01	–
Chaitophorinae	<i>Sipha maydis</i> Passerini	0.07	–
Thelaxinae	<i>Thelaxes</i> sp.	0.01	–
Calaphidinae	<i>Therioaphis trifolii</i> (Monell)	0.02	–
Phylloxeridae	<i>Phylloxera</i> sp.	–	0.08
Unidentified (damaged)		3.67	8.51

‰: is the frequency of aphid species captured in YWTs in Douala and El Ogl.

STATISTICAL ANALYSIS

A chi-square test was performed to compare the incidence of PVY, PLRV, PVA, PVS and PVX occurring in ware and seed potatoes in different growing potato areas in two cropping seasons.

RESULTS

DIVERSITY OF APHID TAXONOMY AND SPECIES COMPOSITION

A total of 9966 aphids were captured by yellow water traps during the study period in both Douala and El Ogl. 73 taxa were recorded distributed as 64 taxa identified at species level and 9 taxa at genus level. All the aphids collected belonged to the Aphididae family except for one specimen of Phylloxeridae captured in El Ogl. The Aphidinae subfamily was the most frequent in the Aphididae family (91.6%), however the following subfamilies: Pemphiginae, Lachninae, Eriosomatinae, Chaitophorinae, Thelaxinae and Calaphidinae each constituted 1.4% (Table 1).

The number of taxa increased with mean temperature at both sites, hence positive correlation (r) occurred in Douala and El Ogl ($r=0.8$; $r=0.7$), respectively (Fig. II). Moreover, 24 °C as average temperature corresponded to the highest number of taxa recorded at both sites: 44 in Douala and 22 in El Ogl as shown in figure (Fig. II). Interestingly in terms of abundance, 24°C remained the favourable average of temperature for the maximum number of aphids captured in El Ogl ($N=617$). However, the peak of aphids in Douala ($N=3800$) was associated to $T = 20^{\circ}\text{C}$.

VARIABILITY OF LANDING APHIDS DUE TO SPATIAL AND TEMPORAL FACTORS

The distribution of species depending on region fac-

tors determined around 56% of the total identified species found in Douala. However, 8% of the species were collected only in El Ogl and the remaining species (36%) were collected in both localities (Table 1).

In Douala, in the Cap Bon region, 8768 aphids were captured and *Aphis spiraecola* Patch (66.1%) and *Aphis* spp. group (13.5%) were the most abundant species followed by *Acyrtosiphon pisum* (Harris) and *Aphis fabae* Scopoli which had a very similar percentage of abundance (around 3%). However, except for *Aphis gossypii* (Glover) (1.8%), *Metopolophium dirhodum* (Walker) (1.7%) and *Aploneura lentisci* (Passerini) (1.4%), the remaining species all had a relative abundance of less than 1% (Table 1). At El Ogl, in the Goubellat region, the number of captured aphids was lower, with 1198 specimens recorded of which three species were the most frequent: *Rhopalosiphum padi* (L.) (58.9% of total catches), *A. pisum* (12%) and *A. lentisci* (10.4%). There were lower numbers of *Aphis craccivora* Koch (1.8%) and *Aphis* spp. and *Hyperomyzus lactucae* (L.) (both 1.1%). Some species were found sporadically (Table 1). With regard to potato colonising aphids and their prevalence, it is worth noting the presence of *A. gossypii* exclusively in Douala. The following species occurred in both Douala and El Ogl, respectively: *A. fabae* (3.1% vs. 0.7%), *Myzus persicae* (Sulzer) (0.6% vs. 0.4%) and *Macrosiphum euphorbiae* (Thomas) (0.1% vs. 0.2%) (Table 1).

Regarding temporal variations, in Douala there were fewer winged aphid catches at the beginning of the study but then the total abundance (N) progressively increased from 3 to 252 aphids in the third week of April corresponding to species richness (S) ranged between 3 and 27. Subsequently, a smaller peak occurred in late April ($N=636$; $S=34$). After this, population abundance reached a plateau in the first two

Fig. II - Relationship between mean temperature (°C) and number of taxa recorded in Douala (1) and El Ogla (2).

Fig. III - Panels (1, 2) show the evolution of total winged aphids and contribution of the most frequent species captured in yellow water traps during the potato growing season in Douala (1) and El Ogla (2). Panels (3, 4) depict the weekly abundance of aphids in relation with species richness (S), Shannon Wiener index (H'), Simpson's index (1-D) and their evenness expressed as Pielou's evenness (EH) and Simpson's equitability (ED) in Douala (1) and El Ogla (2).

weeks of May (respectively $N=1762$; $S=40$ and $N=1787$; $S=22$). In the third week of May population peaked suddenly reaching the maximum number of winged aphids ($N=3800$; $S=24$) and declined towards the end of May ($N=382$; $S=19$) (Fig. III, 1, 3). It is of note that the correlation between abundance and species richness in Douala was moderate ($r=0.51$) (Fig. III, 3). In El Ogla, the population dynamic showed one important peak at the beginning of May ($N=617$; $S=21$)

and declined drastically in mid May ($N=161$; $S=15$), hereafter, it stabilised more or less for the remaining period of the study until May 26th corresponding to the date of haulm killing (Fig. III, 2, 4). However, conversely to the Douala site, species richness and aphid abundance followed the same trajectory which implies the occurrence of positive correlation ($r=0.87$) (Fig. III, 4).

Table 2 – Virus screening of potato leaves sampled from growing areas of ware and seed potatoes during the late and main seasons of 2015.

Season	Late				Main			
Crop	Ware potatoes		Ware potatoes		Seed potatoes			
Site	Douala		Soliman		Douala		El Oglá	
Virus	Ni(+)/N	I (%)	Ni(+)/N	I (%)	Ni(+)/N	I (%)	Ni(+)/N	I (%)
PVY	36(+)/112	32.14	72(+)/112	64.28	37(+)/200	18.50	7(+)/200	3.50
PLRV	3(+)/112	2.68	11(+)/112	9.82	0(+)/200	–	0(+)/200	–
PVA	3(+)/112	2.68	0(+)/112	–	0(+)/200	–	1(+)/200	0.5
PVS	0(+)/112	–	0(+)/112	–	0(+)/200	–	0(+)/200	–
PVX	4(+)/112	3.57	2(+)/112	1.78	0(+)/200	–	0(+)/200	–
PVY+PVA	2(+)/112	1.78	0(+)/112	–				
PVY+PVX	2(+)/112	1.78	2(+)/112	1.78				
PVY+PLRV			8(+)/112	7.14				
Total	46(+)/112	41.07	85(+)/112	75.89	37(+)/200	18.50	8(+)/200	4.00

Ni (+): Number of infected plants/N: Total sample tested, I (%): Incidence (%) given by the formula [Number of infected potato plants/Total sample tested]*100.

In terms of vector pressure, of the 59 species identified in Douala and the 28 species in El Oglá, some species contributed remarkably in the peaks of aphid flight activities and had been reported previously to be potential vectors of potato viruses. Although *A. fabae* and *A. pisum* from Douala were present in equal numbers in early season during the first peak on April 30th (17%, N=631), they were not much involved in the following peaks. *A. spiraecola* comprised 15% of the total YWT catches, and hereafter its presence increased from 51 to 81% of the total catches between May 7th and May 14th when the total number of catches was respectively 1762 and 1787. In the last peak (N=3800) on May 21th its relative abundance was still important (79% of total catches). The numbers of the *Aphis* spp. group were second after *A. spiraecola* with 15% and 13% of total catches (Fig. III, 1). In El Oglá, *R. padi* predominated with 71% of the total aphids caught in early May 5th (N=617). Two species: *A. pisum* and *A. lentisci* were the most prevalent species but they contributed little to vector pressure (3%) during the ascending phase in April. Their relative abundance increased at the end of May and both species constituted more than 40% of the total catches (Fig. III, 2). These observations indicate that vector pressure was more important and earlier in Douala than in El Oglá.

At both sites the fluctuation of the Shannon-Wiener index, the Simpson index and their evenness were similar. The range of the Shannon-Wiener index was between 0.23 and 2.58 in Douala and between 0 and 1.70 in El Oglá (Fig. III, 3, 4). Interestingly, by omitting the date of catch on which the number of aphids corresponded to the number of species found, the highest values in Douala were recorded on April 23th (N=252; H'=2.58; S=27; 1-D=0.88) (Fig. III, 3). In El Oglá the maximum of diversity indices occurred on May 12th (N=161; H'=1.70; S=15; 1-D=0.75) (Fig. III, 4).

MONITORING OF THE MAIN POTATO VIRUSES

In order to investigate the variability of virus infections in two cropping seasons, the DAS-ELISA test revealed that out of 112 potato leaves sampled in Douala during late season, 41.1% constitute total infections distributed as 32.1%, 3.6%, 2.7% and 2.7% with PVY, PVX, PLRV and PVA, respectively (Table 2). In the main season the maximum of infection rate recorded in Soliman was 64.3% for the PVY virus followed by 9.8% and 1.8% of infected leaves with PLRV and PVX, respectively (Table 2).

Similarly of 200 potato leaf samples collected from seed potato fields in the main season, 18.5% and 3.5% were infected with PVY in Douala and El Oglá, respectively. Interestingly no infection by the other viruses screened occurred in Douala, although one sample of 0.5% of total samples tested from El Oglá showed infection with PVA. Overall, it is worth noting that none of the samples tested in either crop season exhibited an infection with PVS (Table 2).

PVY was the most prevalent virus in all the season crops and sampling sites with a maximum of incidence recorded in ware potatoes sampled in Soliman and the lowest rate occurred in seed potato from El Oglá. PLRV and PVX were detected only on ware potatoes collected in the late and main seasons, with PLRV incidence increased from 3 infected samples to 11 respectively. Conversely PVX incidence decreased from 4 samples to 2. PVA was detected in 3 samples of ware potatoes and just in one sample of seed potato. Overall, despite their variability in incidence rates PLRV, PVA and PVX displayed similar prevalence (Table 2).

Regardless of spatial and temporal variations, 28.2% in a total of 624 samples tested reacted positively with antibodies of the five main viruses. PVY presented the highest total incidence (24.4%). PVA exhibited the lower total incidence (0.6 %) followed by PVX (2%)

and PLRV (2.24%) (Table 2).

Mixed infections were detected only in samples of ware potatoes: 3.6% in the late and 8.9% in the main season. PVY was present in all combinations, with equal incidence rates 1.8% recorded in the following co-infections PVY+PVA, PVY+PVX and 7.14% for PVY+PLRV. However, co-infection with PVY+PVX was the most prevalent (Table 2).

Overall the Chi-square test showed significant differences between infection rates in three growing areas (Douala, Soliman and El Ogla) and two cropping seasons (ware potatoes of the late and main seasons and seed potatoes of the main season) ($\chi^2 = 23.22$; $df = 9$; $P = 0.006$).

Comparative analysis of virus incidence occurring in ware potatoes between the main and late seasons revealed a significant difference ($\chi^2 = 9.47$; $df = 3$; $P = 0.024$). Similarly, significant differences were observed in the main season between seed potatoes in Douala and ware potatoes in Soliman ($\chi^2 = 6.33$; $df = 2$; $P = 0.042$) and between seed potatoes in the main season and ware potatoes of the late season from Douala ($\chi^2 = 9.14$; $df = 3$; $P = 0.027$).

DISCUSSION

APHIDOFAUNA OF POTATO FIELD SURVEY

The study of aphidofauna in two sites of potato seed production in Tunisia revealed that the significant species richness observed both in Douala and El Ogla sites is undoubtedly due to the richness of the surrounding vegetation. Biological features and aphid life cycles contribute to this richness. In fact 64% and 71% of identified species from Douala and El Ogla were heteroecious in comparison with monoecious species that accounted for 36% and 29%, respectively.

Of a total of 59 species successfully identified in Douala during ten weeks of study in 2015, 42 species had been previously reported (BOUKHRIS-BOUHACHEM *et al.*, 2007). 17 species were newly found in this study and contributed to the knowledge of Douala aphidofauna. Moreover, unreported subspecies from *A. fabae* group were identified for the first time in Douala yellow water trap: *A. f. Cirsiiacantoidis*, *A. f. mordwilkoii* and of *A. f. solanella*. These sub-species had been reported previously in host plants in northern and coastal areas of Tunisia (BEN HALIMA-KAMEL, 2012; BÉJI *et al.*, 2013). Although *Aphis* spp. group was often within the prominent vector found in Douala YWT, morphological identification at species level still constitutes a challenge that merits much more investigation in further research.

In this research YWTs were used first to study the population dynamic of aphids and their abundance in two potato growing areas in Tunisia. Second, to assess aphid diversity depending on spatial (two areas of seed potato) and temporal (according to date of catches) variations, the Shannon-Wiener index and the Simpson index were used and resulted good estimators as their values depended essentially on the total abundance, species richness and relative abundance of each species

(VUČETIĆ *et al.*, 2013). Altitude and climatic conditions could be associated with the fluctuation observed in diversity indices reported (VUČETIĆ *et al.*, 2013)

VIRUS INCIDENCE IN SEED AND WARE POTATOES

The investigation of ware potato plants sampled from Douala and Soliman by serological test determined the predominance of PVY amongst the five main viruses with 48.2% (108/224) of infections occurring in the late and main seasons, of which 14 samples out of 224 were found to be co-infected. The total infections of PLRV were 6.3% (14/224), 2.7% (6/224) with PVX and 1.3% (3/224) of PVA. Our findings are consistent with previous results which showed the prevalence of PVY in different Tunisian regions where commercial potatoes are cultivated. Hence, of the total of 184 potato leaves sampled in Northern Tunisia 90%, 7%, 5%, 4% and 3% were infected with PVY, PVS, PLRV, PVX and PVA respectively (DJILANI-KHOUADJA *et al.*, 2010). In central Tunisia analysis of 201 leaves revealed infection rates of 71% for PVY, likewise PLRV, PVA, PVS and PVX shared the same incidence rate 2% (LARIBI *et al.*, 2012). Our data showed a maximum rate of infection with PVY in the late season (64.3%), similar to a recent study conducted in 14 fields of commercial potatoes in Algeria, showing the occurrence of a maximum infection with PVY 93% in the late season versus a lower incidence of the five main potato viruses, as 5.5% of infections recorded with PLRV and 0.5% for each of PVA, PVS and PVX. Interestingly mixed infections occurred in 39 samples out of 238 on which PVY was present (ALLALA-MESSAOUDI *et al.*, 2018). Our results point out the occurrence of PVY and give evidence of its predominance that is commonly reported in Europe (VALKONEN, 2007) and expected in other countries of Middle East as most seed used to produce table potatoes is imported from the same source as Tunisia i.e. Syria (CHIKH-ALI *et al.*, 2006), Egypt (EL ABSAWY *et al.*, 2012; ELWAN *et al.*, 2017) and Saudi Arabia (CHIKH-ALI *et al.*, 2016).

The infection rate of PVY recorded in the late season was almost twice the infection occurring in the main season (Table 2). We argue that the evolution of PVY incidence over two cropping seasons is due mainly to the use of self-produced seed harvested in the previous main season and serving as plant materials in the late season. In this study conducted in East Africa it is noteworthy that fewer than 4% of farmers in Uganda and fewer than 1% in Kenya used formal seed, things that may hold the key of the highest infection observed with PVY (77%) and consequently only 3% of marketable seed were free from viruses. The authors considered such practices as the main cause of seed degeneration in developing countries (GILDMACHER *et al.*, 2009). Another hypothesis which may explain the high level of PVY observed in all the sites of Cap Bon, is the availability of different inoculum sources. Firstly, *Potato virus Y* (PVY), is the species member of the *Potyviridae* family known to infect a wide host range, especially solanaceous crops (i.e. tomato, pepper, tobacco and eggplant) identified as a potential source of

PVY inoculum mainly when they are cultivated in the vicinity of potato fields (KERLAN and MOURY, 2006).

Further sources of PVY inoculum are solanaceous weeds, which harbour PVY and serve as reservoir plants for PVY inoculum (WERE *et al.*, 2013). *Solanum elaeagnifolium* Cavanilles is a perennial weed found at three main potato seed growing areas in Tunisia and as well as its capacity to maintain and carry PVY infection up to 39%, it promotes PVY spread by hosting colonies of *M. persicae* and *A. fabae* (BOUKHRIS-BOUHACHEM *et al.*, 2007b). However the lower incidence of viruses occurring in the late season is probably due to the absence of alternate hosts mainly for holocycle aphids coinciding with increased temperatures that weaken aphid flight activity.

Regarding seed potato fields, our results showed an unequal distribution of PVY infections in Douala and El Ogla. The latter exhibited a weak incidence rate, less than 5%. However Douala displayed an important infection rate by PVY (18.5%) (Table 2). These data are quite similar to the infection rate by PVY (19%) that occurred in Douala in 2002, however, fluctuation of PVY infection was observed in two consecutive years from 12% in 2005 to 14% in 2006 (BOUKHRIS-BOUHACHEM *et al.*, 2010).

The absence of infections by PLRV, PVA, PVS and PVX similarly from Douala and El Ogla (except for one sample picked in El Ogla with PVA infection), can almost certainly be attributed to the origin and quality of seeds. In fact the quantity of seed potato planted in the main season was entirely imported as Super Elite or Elite classes with virus tolerance ranging between 0.5 and 2%.

Interestingly, the absence of PLRV infections in parcels of seed potatoes could be attributed to roguing. Symptoms of PLRV are widely recognised by visual inspection, and consequently plants carrying symptoms were probably removed by farmers before our sampling. However, it is not sufficient to rely only on roguing because visual inspections can miss symptomless viruses or the response of latent viruses as has been suggested by (ROBERT *et al.*, 2000). Therefore, through agronomic practises such as soil management, resistant cultivar use could minimise infection by PVY (HALTERMAN *et al.*, 2012).

OCCURRENCE OF FLYING VECTORS IN RELATION TO VIRUS SPREAD

In our study the evaluation of Douala YWT content focuses on abundant species, because of their direct implication in virus vectoring. Transient aphid *A. spiraeicola* was the most important species caught. The large observed number of specimens of this species is likely associated with the availability of alternating hosts, mainly *Citrus* orchards located near potato fields, resulting in reduced migration movements. On the other hand a temporal shift in population growth of this species was observed from 4.8% of total catches reported by BOUKHRIS-BOUHACHEM *et al.* in 2007 to 66.1% in the present survey.

Moreover, by tracking the movement of *A. spiraeicola*, its flight activity was earlier in the season and con-

tinued during all the stages of potato growth. According to literature data time of aphid flight activity is the most relevant factor in the epidemiology of PVY (KIRCHNER *et al.*, 2011). The same authors argued that the population peak of *A. fabae* before potato flowering was one of the main reasons that makes *A. fabae* the most important vector of PVY in Finland (KIRCHNER *et al.*, 2011).

This is in line with a recent study conducted in Switzerland determined that only predicting model using the cumulative abundance of *Brachycaudus helichrysi* (Kaltenbach) showed to have a greater impact on PVY outbreaks. However, in the same study *M. persicae* showed unexpected low frequency and late flight activity at the time of plant resistance (STEINGER *et al.*, 2015). Similarly, in our survey, *M. persicae* counts were very low. Nevertheless, previous experiments determined that, of fourteen species tested for their relevance in virus transmission *M. persicae*, *A. spiraeicola* and *A. gossypii* were the most efficient vectors of PVY in Tunisia while *R. padi* was classified as a poor vector (BOUKHRIS-BOUHACHEM *et al.*, 2011).

Building on our findings, we cannot precisely define the impact of vector activity on virus transmission in the field but by combining results from the DAS-ELISA test and YWTs data it can be deduced that: (i) the long lasting flight activity of non-colonising species (i.e. *A. spiraeicola* and group of *Aphis* spp.) captured in large number (ii) the occurrence of flying vectors (i.e. *A. gossypii* and *M. persicae*) previously linked with their highest level of PVY transmission in Tunisia are the major factors contributing to the classification of Douala as a high risk area for the production of healthy tubers.

On the other hand the limited PVY outbreaks observed in El Ogla might be explained by the characteristics of the area intended to seed potato production. El Ogla is geographically isolated and different from Douala which has a varied agricultural landscape (horticultural crops, trees, wild forest, wind breaks) near the cultivated potato fields. Evidently, this confined area (El Ogla) negatively affects the horizontal transmission of PVY inoculum by insect vectors. Moreover, it appears that vector pressure can be variable with altitude as the lower the altitude the higher was the aphid density reported (VUČETIĆ *et al.*, 2013). Literature data are in accordance with our findings as Douala has an altitude of 74 m compared with El Ogla which is situated at 127 m above sea level, corresponding to 8768 specimens caught versus 1198, respectively.

In addition, other factors like aphid phenology and environmental conditions could be associated with vector abundance (KIRCHNER *et al.*, 2013). El Ogla site was surrounded by cereal crops, therefore, *R. padi* was the most preponderant species in this area. *R. padi*, has a heteroecious holocycle, alternating between a woody host *Prunus padus* as primary host and several species of Gramineae as secondary hosts; furthermore, it is viewed as the main vector of the barley yellow dwarf virus (BYDV) (BLACKMAN and EASTOP, 2000). Even if *R. padi* abundance was significant at El Ogla site, its ability to transmit PVY was low (BOUKHRIS BOU-

ACHEM *et al.*, 2011). Features of El Ogla site, including mainly the low efficiency of PVY vector, render El Ogla a suitable area for seed potato production in Tunisia.

CONCLUSION

The diversity of aphids in Douala from the Cap bon region was previously investigated between 2002 and 2004. We aimed in this study to characterise the new pattern of aphid flight activities and species composition of Douala in comparison with El Ogla, a new area of seed potato production. The total cumulative abundance recorded in Douala was much higher than in El Ogla. This was found to be linked to its agricultural landscape and climatic conditions. In order to determine the know-how to establish and to protect fields mostly destined for seed potato production, our data from YWTs elucidate clearly the earlier flight activity coupled with the important abundance of aphid vectors occurring in Douala. This might explain the prevalence and highest incidence of PVY amongst the five main viruses in both ware and seed potatoes. However, El Ogla exhibited a very low incidence of virus diseases. This is mainly due to the effect of low counts of efficient vectors. This area seems to be safe and suitable for the production of healthy potato seeds.

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REFERENCES

- ABDALLA O.A., ERAKY A.I., MOHAMED S.A., FAHMY F.G., 2016 – *Molecular identification of viruses responsible for severe symptoms on potato (Solanum sp.) growing in Assiut governorate (Upper Egypt)*. - International Journal of Virology Studies and Research, 4: 9-33.
- ALLALA-MESSAOUDI L., GLAIS L., KERKOU D., BOUKHRIS-BOUHACHEM S., BOUZNAD Z., 2018 – *Preliminary characterization of Potato Virus Y (PVY) populations in Algerian potato fields*. - Journal of Plant Pathology, 101: 1-14.
- BEN HALIMA-KAMEL M., 2012 – *Aphid fauna (Hemiptera Aphididae) and their host association of Chott Mariem, coastal area of Tunisia*. - Annals of Biological Research., 3: 746-754.
- BÉJI B., BOUKTILA D., MEZGHANI-KHEMAKHEM M., BOUKHRIS-BOUHACHEM S., MAKNI M., MAKNI H., 2013 – *Genetic structure of Aphis fabae Scopoli (Hemiptera, Aphididae) in Tunisia, inferred from RAPD markers*. - Romanian Agricultural Research, 30: 307-315.
- BLACKMAN R.L., EASTOP V.F., 2000 – *Aphids on the world's crops: an identification and information guide*. 2nd Ed. John Wiley & Sons, Chichester, UK.
- VAN EMDEN H.F., HARRINGTON R., 2017 – *Aphids as crop pests*. 2nd Ed., CAB International, London, UK, pp. 30-65.
- BOQUEL S., GIORDANENGO P., 2011 – *Divergent effects of PVY-infected potato plant on aphids*. - European Journal of Plant Pathology, 129: 507-510.
- BOUKHRIS-BOUHACHEM S., SOUSSI R., TURPEAU E., ROUZÉ-JOUAN J., 2007 – *Aphid (Hemiptera: Aphidoidea) diversity in Tunisia in Relation to seed potato production*. - Annales de la société entomologique de France, 43: 311-318.
- BOUKHRIS-BOUHACHEM S., HULLÉ M., ROUZÉ-JOUAN J., GLAIS L., KERLAN C., 2007b – *Solanum elaeagnifolium, a potential source of Potato Virus Y (PVY) propagation*. - EPPO Bulletin, 37: 125-128.
- BOUKHRIS-BOUHACHEM S., GLAIS L., TRIBODET M., KERLAN C., 2010 – *Incidence and characterization of Potato Virus Y in seed potatoes in Tunisia*. - Potato Research, 53: 151-166.
- BOUKHRIS-BOUHACHEM S., ROUZÉ-JOUAN J., SOUSSI R., GLAIS L., HULLÉ M., 2011 – *Transmission efficiency of the strain PVY NTN by commonly captured aphids in Tunisian potato fields*. - Plant Pathology, 10: 22-28.
- BRUNT A.A., 2001 – *The main viruses infecting potato crops*. In: Virus and virus-like diseases of potatoes and production of seed-potatoes, Loebenstein G., Berger P.H., Brunt A.A., Lawson R.H. Ed., Kluwer, Academic Publishers, Dordrecht, pp. 65-67.
- CHERIF C., BOUDHIR H., 1990 – *Etude épidémiologique des Virus Y et enroulement de la pomme de terre dans le Nord-Est de la Tunisie*. - Annales de l'Institut National de la Recherche Agronomique de Tunisie, 63 :1-24.
- CHERIF C., MNARI-HATTAB M., 1994 – *Contribution à l'étude du virus Y de la pomme de terre*. - Annales de L'Institut National de la Recherche Agronomique de Tunisie, 67: 21-30.
- CHIKH-ALI M., KATAYAMA K., MAOKA T., NATSUAKI T.K., 2006 – *Occurrence of Potato Virus Y on potato in Syria*. - Japanese Journal of Tropical Agriculture, 50: 23-28.
- CHIKH-ALI M., ALRUWAILI H., VANDER POL D., KARASEV A.V., 2016 – *Molecular characterization of recombinant strains of Potato Virus Y from Saudi Arabia*. - Plant disease, 100: 292-297.
- CLARK M. F., ADAMS A.N., 1977 – *Characteristics of the microplate method of Enzyme-Linked Immunosorbent Assay for the detection of plant viruses*. - Journal of General Virology, 34: 475-483.
- DJILANI-KHOUDJA F., GLAIS L., TRIBODET M., KER-

- LAN C., FAKHFAKH H., 2010 – *Incidence of potato viruses and characterisation of Potato Virus Y variability in late season planted potato crops in Northern Tunisia*. - European Journal of Plant Pathology, 126: 479-488.
- ELWAN, E. A., ABDEL-ALEEM E.E., FATTOUH F.A., 2017 – *Occurrence of diverse recombinant strains of Potato Virus Y circulating in potato fields in Egypt*. - Plant Disease, 101: 1463-1469.
- EL-ABSAWY E.A., MAHMOUD A., HEMEIDA A.A., HELMY M., 2012 – *Molecular variation of Potato Virus Y isolated from Egypt*. - International Journal of Virology, 8: 81-89.
- GILDEMACHER P.R., DEMO P., BARKER I., KAGUONGO W., STRUIK P.C., 2009 – *A description of seed potato systems in Kenya, Uganda and Ethiopia*. - American Journal of Potato Research, 86: 373-382.
- HALTERMAN D., CHARKOWSKI A., VERCHOT J., 2012 – *Potato, viruses, and seed certification in the USA to provide healthy propagated tubers*. - Pest Technology, 6: 1-14.
- JACKY F., BOUCHERY Y., 1984 – *Atlas des formes ailées des espèces courantes de pucerons*. INRA Colmar, 45 pp.
- KERLAN C., 2008 – *Potato viruses*. Encyclopaedia of Virology 5: 296-309.
- KERLAN C., MOURY B., 2008 – *Potato Virus Y*. In: Encyclopaedia of virology 3rd edn, Mahy B.W.J., Van Regenmortel M.H.V. Ed., Elsevier, Oxford, pp. 287-296.
- KHAMASSY N., RIADH I., BOUKHRIS-BOUHACHEM S., 2015 – *Potato yield variation as affected by virus seed degeneration and growth conditions in Tunisia*. In: Potato and sweetpotato in Africa: transforming the value chains for food and nutrition security, Low J., et al. Eds., CAB International, pp. 207-211.
- KIRCHNER S.M., DORING T.F., HILTUNEN L.H., VIRTANEN E., VALKONEN J.P.T., 2011 – *Information theory-based model selection for determining the main vector and period of transmission of Potato Virus Y*. - Annals of Applied Biology, 159: 414-427.
- KIRCHNER S.M., HILTUNEN L., DORING T.F., VIRTANEN E., PALOHUHTA J.P., VALKONEN J.P.T., 2013 – *Seasonal phenology and species composition of the aphid fauna in a northern crop production area*. - PLoS One, 8: 1-12.
- KOSTIW M., 1975 – *Investigation on the retention of Potato Viruses M and Y in two species of aphids (Myzus persicae Sulz. and Aphis nasturtii Kalt.)*. - Potato Research, 18: 637-640.
- LACOMME C., PICKUP J., FOX A., GLAIS L., DUPUIS B., STEINGER T., ROLOT J.L., VALKONEN J.P.T., KRUGER K., NIE X., MODIC S., MEHLE N., RAVNIKAR M., HUILLÉ M., 2017 – *Transmission and epidemiology of Potato Virus Y*. In: Lacomme C. et al. Eds., Potato virus Y, biodiversity, pathogenicity, epidemiology and management, Springer, Dordrecht, the Netherlands, pp. 141-177.
- LARBI I., DJILANI-KHOUDJA F., KHAMASSY N., FAKHFAKH H., 2012 – *Potato virus surveys and wide spread of recombinant PVY^{NTN} variant in central Tunisia*. - African Journal of Microbiology Research, 6: 2109-2115.
- MAGURRAN A.E., 2004 – *Measuring biological diversity*. Oxford, Blackwell Science, 256 pp.
- MOERICKE V., 1955 – *Über die Lebensgewohnheiten der Geflügelten Blattlaus (Aphidina) unter besonderer Berücksichtigung des Verhaltens beim Landen*. - Zeitschrift für Angewandte Entomologie, 37: 29-91.
- NAULT, L.R., 1997 – *Arthropod transmission of plant viruses: a new synthesis arthropod transmission of plant viruses*. - Annals of the Entomological Society of America, 90: 521-541.
- PIELOU E.C., 1969 – *An introduction to mathematical ecology*. John Wiley and Sons, New York. X + 294 pp.
- RADCLIFFE E.B., RAGSDALE D.W., 2002 – *Invited review aphid-transmitted potato viruses: the importance of understanding vector biology*. - American Journal of Potato Research, 79: 353-386.
- REMAUDIÈRE G., REMAUDIÈRE M., 1997 – *Catalogue of the world's Aphididae (Homoptera Aphidoidea)*. INRA Edition, Paris, 473 pp.
- ROBERT Y., WOODFORD T.J.A., DUCRAY-BOURDIN D.G., 2000 – *Some epidemiological approaches to the control of aphid-borne virus diseases in seed potato crops in northern Europe*. - Virus Research, 71: 33-47.
- SHANNON C.E., WEAVER W., 1964 – *The Mathematical Theory of communication*. The University of Illinois press, Urbana, IL. 117 pp.
- SIMPSON E.H., 1949 – *Measurement of Diversity*. - Nature, 163: 688.
- STEINGER T., GOY G., GILLIAND H., HEBEISEN T., DERRON J., 2015 – *Forecasting virus disease in seed potatoes using flight activity data of aphid vectors*. - Annals of Applied Biology, 166: 410-419.
- VALKONEN J.P.T., 2007 – *Viruses: economical losses and biotechnological potential*. In: Potato biology and biotechnology: advances and perspectives, Vreugdenhil D., Bradshaw J., Gebhart C., et al. Eds., Elsevier, Oxford, UK, pp. 619-641.
- UČETIĆ A., VUKOV T., JOVIČIĆ I., PETROVIĆ-OBRADOVIĆ O., 2013 – *Monitoring of aphid flight activities in seed potato crops in Serbia*. - ZooKeys, 319: 333-346.
- WERE H.K., KABIRA J. N., KINYUA Z. M., OLUBAYO F. M., KARINGA J. K., AURA J., LEES A. K., COWAN G. H., L. TORRANCE L., 2013 – *Occurrence and distribution of potato pests and diseases in Kenya*. - Potato Research, 56: 325-342.