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COTTON RACES IN APHIS GOSSYPII EVIDENCED BY MICROSATELLITE MARKERS AND LIFE HISTORY TRAITS

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Abstract

The cotton aphid *Aphis gossypii* Glover is key pest of cotton in northern Cameroon, particularly at early crop stages. The objective of this work was to evaluate the clonal diversity and host specificity of aphids' populations infesting cotton. To accomplish this task, samples were collected at different periods, in different sites and from different host plants in the cotton-growing area of Cameroon. The genetic structure of collected samples was screened using microsatellite markers while demographic parameters of cotton aphids according to host plant were assessed in laboratory bioassays.

Very low clonal diversity was observed in *A. gossypii* populations, as only three clones were detected in the samples originating from cotton. During the dry season, the major clone (*Burk)* was found principally in cultivated malvaceous plants such as roselle and okra that constitute excellent relay host plants. In addition, the intrinsic rate of population growth (r_m) of this clone was found to be equal on okra (0.32) but significantly greater on roselle (0.42), compared to cotton. On the contrary, *Burk* was rarely found in aphid colonies collected from Cucurbitaceae and Solanaceae, and antibiosis tests confirmed that reproductive potential was negatively affected on melon (0.22) and sweet pepper (0.00). These results confirm that host plant specificity acts in genetic structuring of aphid populations, with particular emphasis on cotton aphids.

Introduction

Aphis gossypii Glover is a cosmopolitan and polyphagous species widely distributed in tropical, subtropical and temperate regions. Because aphids have a high rate of increase due to high fecundity and overlapping generations, they have the potential to rapidly colonize plants. This species is key pest of cotton in Cameroon. As a sap feeder and virus vector, it may directly affect plant health, particularly at early crop stages. During late season, it represents a strong threat to fiber quality, by honeydew contamination on open bolls (sticky cotton).

Recently, molecular markers showed the existence of host races on Cucurbitaceae, cotton, eggplant, pepper and sweet pepper (Vanlerberghe and Chavigny, 1998; Carletto, 2004). According to the same approach, the present study was carried out to assess the genetic diversity of *A. gossypii* populations in the cotton- growing area of northern Cameroon. Which are the genotypes that infest cotton fields throughout the season? Are these populations composed of generalist clones that colonize a large range of plants, or composed of cotton-specific clones? Which are the alternative host plants during the dry season (December to mid-May)? For integrated pest management, understanding genetic diversity and dynamics of *A. gossypii* populations could serve to explain breakouts and recommend adequate tools for monitoring and control.

Materials and methods

Genetic analyses of population samples

In 2003 and 2004, two non-treated cotton fields in Djalingo (9°23' N, 13°45' E; 2400 m²) and Kodek (10°66' N, 14°41' E; 2000 m²) were inspected for aphid's collection. Each year, individuals were collected three times during the growing season: at the beginning of infestation (first detected individuals), at the peak of early infestation and at the peak of late season infestation. At the beginning of infestation, 40 to 50 plants hosting

some individuals were marked. Apterous adults from each "newly infested plant" were individually transferred in 1.5 ml Ependorf tubes. Subsequent samplings were carried out on the same marked plants.
Additional samplings on various cultivated host plants (Solanaceae, Cucurbitaceae and Malvaceae) were carried out during the dry season, in irrigated areas near Garoua (9°31' N, 13°40' E) and Maroua (10°60' N, 14°32 E). For a given plot, 40 apterous adults were individually collected near the peak of infestation on 40 non-neighboring plants.

Approximately 800 individuals were collected and genotyped at each of the 8 microsatellite loci identified for *A. gossypii* (Vanlerberghe-Masutti *et al.,* 1999). Polymorphism corresponded to allele's size at a given locus, based on the number of nucleotide chain replications. For DNA extraction, aphids were washed twice in a NaCl 0.65 % (w/v) solution, before crushing in a 0.5 ml tube containing 55 µl of 5% Chelex (w/v). Each tube was immersed in a hot water basin at 56°C for 30 minutes followed by 100°C for 7 minutes. The extracts were then vortexed, centrifuged and conserved at -20°C.

DNA amplification by multiplex-PCR and microsatellite analyses was performed at INRA of Sophia-Antipolis (UMR INRA-1112-UNSA ROSE). Variations in allele's size were visualized by electrophoresis in an automatic sequencer ABI 3100 Genetic Analyzer. The results were analyzed by STRAnd (Nucleic Acid Analysis Software, http://www.vgl.ucdavis.edu/informatics/STRand). Each individual was assigned a multilocus genotype representing a combination of the 8 loci. Individuals characterized by one similar multilocus genotype were considered as belonging to the same clone (Carletto, 2004).

Life history traits assessment

The cotton clone *Burk* was established from apterous cotton aphids collected near Garoua (Cameroon) in 2005 and maintained on potted cotton plants in mesh cages, with glass covers. All experiments were carried out in a climatic chamber at 25±2°C and 12 h of artificial light. For antibiosis bioassays, 60 apterous adults were randomly collected from a *Burk* clone colony. Two aphids were transferred on a leaf disc of the tested plant (30 replicates), placed upside down on gel (5% agar-agar) in Petri dishes of 2 cm diameter. After 24 hours, emergent nymphs were counted and the adults removed. After 48 hours, live descendants were counted and removed, except one. Thereafter, each Petri dish was inspected daily to monitor the aphids' development, survival and fecundity. During the reproduction period, nymphs were counted daily and immediately removed. To prevent nutritional deficiency, every 3-4 days each aphid was transferred to a new leaf using a fine tipped camel-hair brush.

Burk demographic parameters were assessed on control cotton (*Gossypium hirsutum* L.) *versus* okra (*Abelmoschus esculentus* [L.] Moench), roselle (*Hibiscus sabdariffa* L.), melon (*Cucumis melo* L.), eggplant (*Solanum melongena* L.), sweet pepper (*Capsicum annuum* L.) and cassava (*Manihot utilissima* Pohl) or bougainvillea (*Bougainvillea glabra* Choisy), occasional and non- host plants, respectively, for *A. gossypii.*

Population growth parameters were estimated with the formulae provided by Birch (1948). The intrinsic rate of increase (r_m) was calculated by iteratively solving the equation $\sum l_x m_x e^{-rx} = 1$, where the age-specific survival rate (l_x) is the proportion of individuals in the original cohort alive at age x, and the age-specific fecundity (m_x) is the mean number of female progeny produced per life female in the age-interval x. The results were analyzed using the Student test based on jackknife estimates of variance (Meyer *et al.*, 1986; Hulting *et al.*, 1990). Differences among two treatments were assessed with the following equations:

$$LSR = Q_{\alpha[2,v]} \sqrt{S^2_{av}} \sqrt{\frac{n_i+n_j}{2n_in_j}} \quad \text{and} \quad S^2_{av} = \frac{\sum (n_i-1)S_i^2}{\sum (n_i-1)},$$

where n_i and n_j were sample sizes of the two compared treatments, $Q_{\alpha[2,v]}$ the value from the studentized range, S^2_{av} the weighted average variance, where n_i was the sample size corresponding to the i^{th} value and S^2_i the jackknife estimate of the variance for the i^{th} value (Wang *et al.*, 2001).

Results

Genetic diversity of aphids collected on rainy season cotton

Only three multilocus genotypes were observed in cotton plots. These genotypes, named *Burk*, *Burk 2* and *Ivor* (Tab. 1), were previously found in cotton (Carletto, 2004).

Table 1. Identification of multilocus genotypes assessed by allele combination at 8 microsatellite loci.

Multilocus genotype (clone)	**Ago24**	**Ago53**	**Ago59**	**Ago66**	**Ago69**	**Ago84**	**Ago89**	**Ago126**
Burk	153-155	116-116	163-211	145-152	107-115	118-118	154-160	166-176
Burk 2	153-155	113-116	163-211	145-152	107-115	118-118	154-160	166-176
Ivor	153-153	113-116	161-175	152-154	113-113	104-124	154-154	166-176

Table 2. Relative frequencies of *A. gossypii* clones collected in cotton fields (Djalingo and Kodek, 2003 and 2004 rainy seasons). n = number of individuals.

Year	**Site**	**Genotype**	**Initial infestation**		**Early season peak infestation**		**Late season peak infestation**	
			n	freq.	n	freq.	n	freq.
2003	Djalingo	*Burk*	26	0.76	24	0.73	32	1.00
		Ivor	8	0.24	9	0.27	0	0.00
	Kodek	*Burk*	26	0.92	26	0.96	26	1.00
		Ivor	1	0.04	1	0.04	0	0.00
		Burk 2	1	0.04	0	0.00	0	0.00
2004	Djalingo	*Burk*	35	0.81	35	0.83		
		Ivor	8	0.19	7	0.17		
	Kodek	*Burk*	28	0.90	26	0.87		
		Ivor	3	0.10	4	0.13		

The genetic profile of *Burk* was greatly predominant (0.87) whatever the sampling date, site or year (Tab. 2). Generally, the genetic profile *Ivor* was less frequent (0.13). Only one isolated individual from *Burk 2* genotype was detected. This genotype differs from the multilocus genotype *Burk* by a mutation at the microsatellite locus Ago53, while the *Ivor* profile presents particular alleles at several microsatellite loci (Tab. 1). The fine observation of genotype by marked plants showed that 63.2% of plants initially colonized by *Ivor* at the beginning of infestation were later substituted by *Burk* at the peak of infestation, while only 13.2% of *Burk* were replaced by *Ivor*.

Genetic diversity of aphids collected on dry season host plants

During the dry season, cotton clones (particularly *Burk*) were found with high frequencies on Malvaceae okra and roselle (Tab. 3). On the contrary, their frequency was low on Cucurbitaceae or Solanaceae plants, except one sample in the local variety of eggplant. Non-cotton clones belonged to other *A. gossypii* host races such as *C9* or *C11* (Cucurbitaceae), *Auber* (eggplant) or *Pipo* (pepper and sweet pepper), previously identified by Carletto (2004).

Table 3 Relative proportions of *A. gossypii* cotton clones collected on vegetables in the dry seasons from 2003 to 2005.

Family	Host plant	Site	Sampling date	n	Clone frequency			
					Burk	*Ivor*	*Burk 2*	non-cotton clones
Malvaceae	okra *Abelmoschus esculentus* (L.) Moench	Gaschiga	11/02/04	31	1.00			
			05/02/05	32	0.94			0.06
		Dakar	16/05/03	31	0.97			0.03
		Meskine	11/03/05	32	1.00			
	roselle *Hibiscus sabdariffa* L.	Gaschiga	03/02/04	32	0.97	0.03		
			05/02/05	62	0.87	0.07		0.06
Cucurbitaceae	watermelon *Citrullus lanatus* (Thunb.)	Gaschiga	25/02/05	32				1.00
	melon *Cucumis melo* L.	Gaschiga	25/02/05	33				1.00
		Pitoa	03/03/05	34	0.03			0.97
	cucumber *Cucumis sativus* L.	Pitoa	03/03/05	33				1.00
Solanaceae	eggplant *Solanum melongena* L.	Gaschiga	25/02/04	31	0.90			0.10
			05/02/05	31				1.00
	sweet pepper *Capsicum annuum* L.	Gaschiga	06/01/04	30				1.00
			05/02/05	29	0.07			0.93
	black nightshade *Solanum nigrum* L.	Boklé	01/04/05	36	0.08	0.22		0.70

Demographic parameters

Some common, occasional and non-host plants were compared to cotton for their capacity to host *Burk* cotton clone. The intrinsic rate of population growth (r_m) of the cotton race *Burk* was significantly greater on roselle, equal on okra and eggplant, and significantly lower on melon than it was for aphids reared on cotton (Tab. 4). Both adult longevity and total progeny production significantly increased when *Burk* clones fed on roselle, but these parameters were not different on okra or eggplant. *Burk* aphids showed lower propensity to reproduce on melon despite normal longevity. Development on sweet pepper, cassava and bougainvillea was unsuccessful.

Table 4. Demographic parameters (±se) of *A. gossypii* cotton clone *Burk* on different host plants.

Family	Plant	Intrinsic rate of increase (±se)		Adult longevity (±se)		Mean reproductive rate (±se)		n
Malvaceae	roselle	0.42 ± 0.01	*	25.2 ± 1.5	**	52.3 ± 3.9	*	*29*
	Control	*0.36 ± 0.02*		*15.4 ± 2.0*		*36.5 ± 5.6*		*26*
	okra	0.32 ± 0.01		16.0 ± 1.1		26.8 ± 2.3		*26*
	Control	*0.32 ± 0.01*		*17.1 ± 0.9*		*30.2 ± 4.3*		*27*
Cucurbitaceae	melon	0.22 ± 0.01	**	16.0 ± 1.4		15.3 ± 1.8	**	*30*
	Control	*0.36 ± 0.01*		*17.9 ± 1.8*		*36.6 ± 4.1*		*26*
Solanaceae	eggplant	0.34 ± 0.01		20.2 ± 1.6		37.9 ± 3.9		*30*
	Control	*0.36 ± 0.02*		*15.4 ± 2.0*		*36.5 ± 5.6*		*26*
	sweet pepper	0.00	**	3.1 ± 0.4	**	0.0	**	*25*
	Control	*0.40 ± 0.01*		*19.0 ± 1.6*		*37.6 ± 3.2*		*28*
Euphorbiaceae	cassava	0.00	**	1.0	**	0.0	**	*4*
	Control	*0.38 ± 0.01*		*19.5 ± 1.8*		*35.7 ± 2.8*		*28*
Nyctaginaceae	bougainvillea	0.00	**	8.2 ± 2.0	**	0.7 ± 0.3	**	*10*
	Control	*0.38 ± 0.01*		*19.5 ± 1.8*		*35.7 ± 2.8*		*28*

(** for P<0.01 and * for P<0.05).

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Discussion

Low clonal diversity in cotton

The genetic analyses of *A. gossypii* populations sampled in two cotton plots 250 km distant (Djalingo and Kodek), and during 2 successive years, revealed a low genetic polymorphism since only two clones, *Burk* and *Ivor,* were detected. The presence of a particular clone at a given moment may represent only a stochastic event. However, *Burk* and *Ivor* were previously identified in cotton fields from Burkina Faso and Ivory Coast, respectively (Vanlerberghe *et al.,* 1999, Carletto 2004). The fact that the same genotypes were found on the same host plants over the years in distant geographic regions suggests a specialization event on this plant. A reduction in clonal diversity was observed during the colonization process in the field, from the very beginning through peak infestation and to the end of the cropping season. This reduction was always associated with the increasing frequency of *Burk*. As a consequence, this clone may have greater fitness on cotton than *Ivor*. Nevertheless, analyses of two samples collected in non-treated, isolated irrigated cotton fields in the dry season (Djarengol, Maroua, 14/03/05) and in wild cotton plants (Ziguinchor, Senegal, at a distance of over 250 km from the cotton-growing zone, 22/04/05) both predominantly revealed the *Ivor* clone with a frequency of 0.95 and 1.00 respectively. In this case, it may be hypothesized that factors such as the absence of insecticide treatments or strong founder effects may interact with the potential interclonal competition (Fuller *et al.*, 1999).

Host specificity

Genetic analysis of aphids' samples collected from different host plants around irrigated areas during the dry season in North Cameroon and antibiosis tests confirmed the existence of cotton, or more widely, Malvaceae host races. The biotype constituents of cotton i.e. *Burk* and *Ivor* were detected in high proportions in samples that were collected from Malvaceae such as okra or roselle, crops that are heavily infested and present the entire year in non-negligible surface areas in North Cameroon (Deguine *et al.*, 1999). Moreover, the intrinsic rate of population growth (r_m) of the cotton clone *Burk,* equal on okra, increased significantly on roselle. These two plants thus constitute an excellent reservoir of aphids in the dry season when cotton hosts are no longer present. Furthermore, a small field of a local variety of eggplant in Gaschiga showed infestation that was largely dominated by *Burk*. On this same glabrous variety, aphids showed good fitness, contrarily to exotic varieties (Picault, unpublished data). In Burkina Faso, the aphid samples collected from 12 eggplant fields did not reveal the cotton clone *Burk* (Carletto 2004). The role of cultivated eggplants as relay plants for the cotton biotypes may result from a founder effect, in the absence of competition with specialized clones. On the contrary, cotton clones were rarely found in aphid colonies collected from Cucurbitaceae and other Solanaceae. Antibiosis tests confirmed that reproductive potential was negatively affected on melon (Cucurbitaceae) or sweet pepper (Solanaceae). On occasional or non-hosts such as cassava and bougainvillea respectively, longevity was drastically reduced and progeny production near zero.

Assessment of demographic parameters of the cotton clone *Burk* in different host plants showed a probable selective barrier participating in host races. Relevant experiences reported differential fitness on different host plants in *A. gossypii* clones (Guldemond *et al.*, 1994; Wool *et al.*, 1995). However, our observations showed that cotton clones were present at the very beginning of cotton infestation. This may indicate that winged forms were capable of selecting the desired host rather than interclonal selection and/or founder effects. Several questions still remain about host selection mechanisms in aphids. After landing as a result of visual stimulation an aphid will pierce a leaf to test for suitability. If the plant is not suitable, it embarks on another flight until it recognizes its host and subsequently settles and multiplies. Experiments on behavioral responses to plants are in progress to evaluate the ability of winged forms to find host plants.

Conclusion

These results demonstrate that host plant specialization acts in genetic structuring in aphid populations, with particular emphasis on cotton aphids, where two major specialized clones may compose the cotton aphid biotype in Cameroon. A broad scale sampling strategy should be conducted to confirm our results. On the other hand, cultivated plants such as okra and roselle constitute excellent off-season reservoirs. From an applied point of view, these results may orientate control measures or resistance management, not for *A. gossypii sensu lato* but more specifically for clones and associated host range. It would be interesting to determine factors that affect the distribution of *Burk* and *Ivor*, by assessing differential host selection ability and fitness on local cotton cultivars or intermediate host plants, according to insecticide treatments and climate. If these preliminary findings allow for better understanding of the cotton aphids, it will be fundamental to complete the identity of individuals or clones with information on alleles resistant to insecticides they carry.

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