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Insecticide use and competition shape the genetic diversity of the aphid *Aphis gossypii* in a cotton-growing landscape

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Abstract

Field populations of the cotton aphid, *Aphis gossypii* Glover, are structured into geographically widespread host races. In the cotton-producing regions of West and Central Africa (WCA), two genotypes have been repeatedly detected within the cotton host race, one of which (*Burk1*) is prevalent (>90%) and resistant to several insecticides, as opposed to the second one (*Ivo*). Here, we conducted whole plant and field cage experiments to test hypotheses for such low genetic diversity, including selection from insecticide treatments, interclonal competition and adaptation to host plant, or climatic conditions. To assess the genetic diversity of immigrant aphids, alatae were trapped and collected on cotton and relay host plants (okra and roselle) in the early cropping season. Individuals were genotyped at eight specific microsatellite loci and characterized by a multilocus genotype (MLG). When independently transferred from cotton (*Gossypium hirustum* L.) leaf discs to whole plants (*G. hirsutum* and *G. arboreum*, roselle and okra), *Ivo* and *Burk1* performed equally well. When concurrently transferred from cotton leaf discs to the same plant species, *Ivo* performed better than *Burk1*, indicating that competition favoured *Ivo*. This was also the case on *G. hirsutum* growing outdoors. Conversely, *Burk1* prevailed when cotton plants were sprayed with insecticides. In experiments where aphids were allowed to move to neighbouring plants, *Burk1* was better represented than *Ivo* on low-populated plants, suggesting that dispersal may be a way to avoid competition on crowded plants. Most cotton aphids collected on cotton or relay host plants in the early cropping season were *Burk1* (>90%), indicating high dispersal ability and, probably reflecting high frequency on host plants from which they dispersed. In the agricultural landscape of WCA, the use of broad-range insecticides on both cotton and relay host plants has led to the prevalence of one genotype of *A. gossypii* resistant to different classes of insecticides. Deployment of widespread and integrated pest management strategies are needed to restore cotton aphid control.

Keywords: selection, dispersal, Aphididae, insecticide resistance, integrated pest management

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Introduction

The cotton aphid *Aphis gossypii* Glover is a cosmopolitan and polyphagous crop pest with more than 600 host species (Inaizumi, 1980; Blackman & Eastop, 1984; Deguine *et al.*, 1997; Ebert & Cartwright, 1997). However, *A. gossypii* populations are structured into host races specialized on Cucurbitaceae, cotton, eggplant, potato, chili- or sweet pepper, and strawberry (Vanlerberghe-Masutti & Chavigny, 1998; Fuller *et al.*, 1999; Brévault *et al.*, 2008; Charaabi *et al.*, 2008; Carletto *et al.*, 2009). As evidenced by the few number of microsatellite multilocus genotypes (MLG) detected, each host race comprises some very specialized genotypes that are geographically widespread and persistent over time through parthenogenetic reproduction (Carletto *et al.*, 2009). In the cotton-producing regions of West and Central Africa (WCA), *A. gossypii* is a key pest of cotton that causes direct damage to seedlings and spoilage of fiber through honeydew production. Over this large geographic area, mainly two genotypes, exhibiting the *Burk1* (>90%) and *Ivo* MLG's, have been repeatedly collected from cotton crops and Malvaceous vegetable crops such as roselle (*Hibiscus sabdariffa* L.) and okra (*Abelmoschus esculentus* (L.) Moench) (Brévault *et al.*, 2008; Carletto *et al.*, 2009). These vegetable crops represent major relay host plants for cotton-adapted genotypes during the off-season when cotton crops are no longer available (Brévault *et al.*, 2008).

Drift and selection, in combination with parthenogenetic reproduction, could both account for the low genotypic variability observed within populations of the cotton host race (Wright, 1969). Brévault *et al.* (2008) showed that the frequency of *Burk1* in cotton crops significantly increased throughout the course of the growing season, especially when cotton fields were sprayed with insecticide. They proposed that selective pressures resulting from the intensification of cotton cultivation and related insecticide treatments have favored *Burk1*-related insecticide-resistant genotypes. A recent study on insecticide resistance traits within and among host races in *A. gossypii*, showed that, in comparison to *Ivo*, *Burk1* was highly resistant to organophosphates (dimethoate, profenofos and monocrotophos), pyrethroids (cypermethrin) and DDT (Carletto *et al.*, 2010). Additional selective factors that could contribute to low genetic diversity and prevalence of *Burk1* include interclonal competition for resources (Fuller *et al.*, 1999; Rochat *et al.*, 1999), ecological specialization on host plants, and superior ability to locate suitable host plants in the spatial and temporal heterogeneity of cotton-based agricultural landscapes (Brévault *et al.*, 2008; Carletto *et al.*, 2009). Adaptation of some genotypes to environmental conditions, such as high temperatures and low relative humidity prevailing during the off-season, could also contribute to low genetic diversity (Vorburger, 2004).

The objective of this study was to identify selective factors that account for the low genetic diversity within the *A. gossypii* cotton host race in WCA and prevalence of *Burk1*. We conducted laboratory and field experiments to assess the relative population growth of the two cotton MLGs, *Burk1* and *Ivo*, in competition or not and according to selective pressures such as host plant, insecticide use and climatic conditions. We also collected *A. gossypii* alatae, both from field traps and cotton plants, early in the cropping season to evaluate the genetic diversity of the immigrant aphid population ('inoculum'). When the cotton season ended, the same type of sampling was done in newly planted plots of okra and roselle, known to act as relay host plants for cotton-specialized aphids throughout the off-season. *A. gossypii* aphids were genotyped at eight specific microsatellite loci and characterized by their MLGs. Results are discussed in the light of interclonal competition within *A. gossypii* field populations and selection by insecticides.

Materials and methods

Insects and multilocus genotype analysis

The two clonal lineages *Burk1* and *Ivo* were originally field-collected from cotton in northern Cameroon (Garoua, 9°23N and 13°45E) in 2006. Their multilocus genotype was established on the basis of the alleles observed at eight microsatellite loci as described by Brévault *et al.* (2008) and Carletto *et al.* (2009). They were raised on cotton leaf discs of 9 cm in diameter (*Gossypium hirsutum* L., cv. Irma A1239, Cameroon) positioned on an agar-coated petri dish (20 g l^{-1} agar and 30 mg l^{-1} nipagin) and held in the laboratory under controlled conditions (25 ± 2°C, 70 ± 20% r.h. and a 14:10 h L:D photoperiod cycle). Aphids that were three days old (4th instar and adult) were used in the experiments. A batch of 10–20 aphids were genotyped at the beginning of each experiment to be sure that there was no contamination. As opposed to *Ivo*, *Burk1* is highly resistant to cypermethrin (pyrethroid) due to the mutation super-kdr (M918L) in the voltage-gated sodium channel gene (para gene), and metabolic detoxification is mediated by esterase enzymes (Carletto *et al.*, 2010). This genotype also carries the mutation A302S in this gene, which confers moderate resistance to organophosphates such as profenofos and monocrotophos (Carletto *et al.*, 2010).

Whole plant experiments

The two clonal lineages *Burk1* and *Ivo* were tested on whole host plants in the laboratory. Tested host plants were cotton (*Gossypium arboreum*, wild type N1301, and *G. hirsutum*, cv. Irma A1239), roselle and okra. The tetraploid cotton *G. hirsutum* probably originated about 1–2 million years ago from an interspecific hybridization of an Old World diploid species that was closely related with *G. arboreum* and a New World diploid species (Beasley, 1940; Wendel & Cronn, 2003). Furthermore, a recent study showed that *G. arboreum* was less susceptible to *A. gossypii* than *G. hirsutum* (Nibouche *et al.*, 2008). Plants were grown in 20-cm diameter pots containing a 50:50 mixture of sand and potting mix. They did not receive any application of fertilizer and were used for experiments four weeks after planting. Cotton seeds were provided by CIRAD cotton germplasm. Four plants were grown in each pot and watered daily. Climatic conditions in the growth chamber were 23–30°C, 45–80% r.h., 12:12 h L:D and 14,000–18,000 lux at plant height.

In experiments 1 to 4, pots were surrounded by an insect-proof mesh to avoid aphid contaminations from neighbouring plants. The treatments in each experiment were replicated eight times and arranged in a randomized block design.

Experiment 1

Host plant (no competition). Four three-day-old apterous *Burk1* or *Ivo* adults were separately transferred from the leaf discs onto four plants of *G. arboreum*, *G. hirsutum*, roselle or okra.

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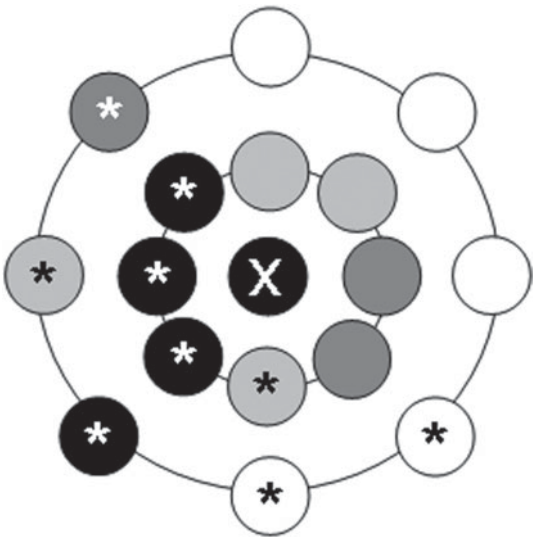

Fig. 1. Density of *Aphis gossypii* genotypes *Ivo* and *Burk1* concurrently inoculated (competition) onto whole cotton plants (*G. hirsutum*) and proportion of *Ivo* after 14 days, experiment 5. X, ten three-day-old apterous *Burk1* and *Ivo* adults were released on d0 on the central plants; *, pots with mean proportion of *Ivo* >50% (□, <250; ▒, 251–500; ▓, 501–750; ■, >750).

Experiment 2

Host plant (competition). The same plant species as in experiment 1 were used; but, here, two three-day-old apterous *Burk1* and *Ivo* adults were concurrently transferred from the leaf discs onto each plant.

Experiment 3

Insecticide use (competition). On d0, two three-day-old apterous *Burk1* and *Ivo* adults were concurrently transferred from the leaf discs to four *G. hirsutum* cotton plants. After ten days, the plants were sprayed with either water or with a mix of commercial formulations of cypermethrin (Cypercal 200 EC, 36 g ha^{-1}) and profenofos (Profenalm 500 EC, 150 g ha^{-1}) according to local cotton pest management recommendations (SODECOTON, 2007).

Experiment 4

Climatic conditions (competition). On d0, two three-day-old apterous *Burk1* and *Ivo* adults were concurrently transferred from the leaf discs to eight *G. hirsutum* plants. Half of the plants were kept in the growth chamber (23–30°C and 45–80% r.h.) and other half were kept outdoors (16–41°C and 20–30% r.h.).

Experiment 5

Genotype dispersion. A total of 16 pots containing four aphid-free *G. hirsutum* plants were placed on two rings around one central pot where ten three-day-old apterous *Burk1* and *Ivo* adults were released at d0 (fig. 1). Aphids were allowed to move from one plant to another.

On d14, the total number of aphids was assessed on each plant. To do this, plants were removed from pots and dipped in 70% ethanol to remove aphids for subsequent counting. To assess the total leaf area of plants, we reproduced leaves on a sheet of paper of known density (g cm^{-2}) and related the resulting paper weight to its area. In experiments 2–5, the number of aphids and proportions of the two clones within the population at d14 were assessed using the discriminating dose of cypermethrin assay. Four batches of 30 individuals were exposed to leaf discs previously sprayed with a dose of 500 mg l^{-1} technical-grade cypermethrin (Arysta Life Science, France) which was assumed to kill only *Ivo* individuals (Carletto *et al.*, 2010), as confirmed by preliminary bioassays where the survivors were genotyped, or to non-treated leaf discs used as controls to evaluate natural mortality.

Field cage experiments

Experiment 6

Field assessment under insecticide pressure. Four insect-proof cages (250 × 250 × 250 cm) were positioned in a 0.25-ha cotton field in the early growing season. Cages contained three rows of ten cotton plants (*G. hirsutum*), each at the pre-flowering stage. On d0, five three-days-old apterous *Burk1* and *Ivo* adults were concurrently transferred on each of two terminal leaves of four randomly chosen plants per cage (resulting in a total of 40 *Burk1* and 40 *Ivo* per cage). Initially, infested plants were tagged with a coloured thread of wool. On d6, a mix of cypermethrin (Cypercal 200 EC, 36 g ha^{-1}) and profenofos (Profenalm 500 EC, 150 g ha^{-1}) was sprayed on plants in two cages with a low volume (10 l ha^{-1}) hand held Ulva+ (Micron Sprayer, UK) sprayer. During application, treated plots were surrounded by a plastic tarpaulin to prevent insecticide drift to neighbouring cages. Control plots were sprayed with water only. Aphids from the five terminal leaves of the four tagged plants and four randomly chosen plants were counted on d14. One aphid was randomly collected on each observed leaf and held at −20°C in 95% ethanol for subsequent MLG analysis.

Field collection of alate aphids

To assess the identity of immigrant aphids (founders) on cotton crops, traps were placed in newly planted cotton fields during the early cropping season (June 2006) to sample alatae. Two sites, about 10 km apart, Djalingo (9°13′N, 13°26′E) and Mayo Dadi (9°15′N, 13°41′E), were selected in the cotton growing area of northern Cameroon. In each site, five traps were placed in a cotton plot and in a neighbouring corn (*Zea maïs* L., non-host plant) field as a control. Traps consisted of sticky yellow cards (Biosystèmes, France) and yellow plates filled with soapy water. Traps were inspected daily. Once some individuals were detected on traps, alatae were also collected on 20 tagged plants per plot. A similar trapping system was implemented in the early off-season (November) to sample alatae on early planted okra or roselle (Malvaceae) in irrigated plots at Gaschiga (9°25′N, 13°21′E) and Pitoa (9°23′N, 13°30′E). Once aphids were detected on traps, alatae were also collected on 20 tagged plants per plot. Aphids were individually observed under a binocular microscope to identify the species according to morphological criteria (Stroyan, 1984). Samples were held at −20°C in 95% ethanol for subsequent MLG analysis.

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Table 1. Mean density after 14 days of *Aphis gossypii* genotypes *Ivo* and *Burk1* independently inoculated (no competition) onto whole plants of various species, experiment 1 (95% confidence limits).

Treatment	Nb aphids plant^{-1}				Nb aphids cm^{-2} leaf			
	Ivo		*Burk1*		*Ivo*		*Burk1*	
G. arboreum	138	(98–196)	104	(74–146)	1.3	(1.0–1.8)	0.9	(0.7–1.3)
G. hirsutum	432	(355–526)	561	(487–647)	3.7	(3.1–4.5)	4.4	(3.8–5.1)
Okra	419	(340–516)	401	(339–475)	5.3	(4.5–6.2)	5.3	(4.7–6.1)
Roselle	476	(395–574)	547	(474–632)	5.6	(4.9–6.6)	7.3	(6.5–8.2)
χ^2_3 (*P*)	54.4 (<0.001)		135.7 (<0.001)		103.1 (<0.001)		227.1 (<0.001)	

Nb, number.

Table 2. Mean density of *Aphis gossypii* genotypes *Ivo* and *Burk1* concurrently inoculated (competition) onto whole plants and proportion of *Ivo* after 14 days as a function of host plant, insecticide use, and climatic conditions, experiments 2–4 (95% confidence limits).

Experiment	Treatment	Nb aphids plant^{-1}		% *Ivo*	
2. Plant species	*G. arboreum*	148	(104–189)	76	(73–78)
	G. hirsutum	413	(349–487)	68	(64–70)
	Okra	601	(523–690)	67	(64–70)
	Roselle	267	(217–329)	62	(59–65)
	χ^2_3 (*P*)	109.8 (<0.001)		43.3 (<0.001)	
3. Insecticide	No insecticide	115	(93–143)	64	(60–67)
	Cyp. + Profeno.	47	(34–66)	10	(8–14)
	χ^2_1 (*P*)	20.9 (<0.001)		346.3 (<0.001)	
4. Climatic conditions	Growth chamber (23–30°C, 45–80% r.h.)	131	(106–163)	71	(67–74)
	Outdoor (16–41°C, 20–30% r.h.)	217	(184–256)	70	(67–73)
	χ^2_1 (*P*)	13.6 (<0.001)		0.2 (0.665)	

Nb, number.

Statistical analyses

In the whole plants and field cage experiments, aphid density was analysed using the Generalized Linear Model (GLM) with a Poisson distribution (count data) and log link. Proportions of aphid genotypes on plants were analysed using the GLM procedure with a binomial distribution and a logit link (JMP® 8.0.1, Sas Institute Inc., Cary, USA). Proportions of alate aphids collected from traps and plants were compared using a conventional χ^2-test.

Results

Population growth on whole plants

When *Burk1* and *Ivo* aphids were transferred separately on whole plants, their population developed significantly better on roselle, okra and cotton *G. hirsutum* than on cotton *G. arboreum* (experiment 1; table 1). Generally, *Burk1* performed as well as *Ivo* on the four plant species. When both MLGs were concurrently transferred on the same plants, the total population developed better on okra than on *G. hirsutum*, roselle, and *G. arboreum* (experiment 2; table 2). The proportion of *Ivo* was >60% on the four host plants. Insecticide spray had a detrimental effect on aphid density and proportion of *Ivo* (experiment 3; table 2). Aphid populations were higher under outdoors conditions (average 28.0°C, 23.2% r.h.) than in the growth chamber (average 24.8°C, 64.3% r.h.) but the proportion of *Ivo* remained unchanged (experiment 4; table 2). When both MLGs were initially released on the same central pot and were allowed to colonize neighbouring cotton plants, the mean density of aphids was greater in the inner ring of pots (712 aphids plant^{-1}) than that in the outer one (405 aphids plant^{-1}) (GLM, $\chi^2_1 = 4.0$, $P = 0.045$) after 14 days. The mean proportions of *Ivo* on plants were 51 and 53% on the inner and the outer rings, respectively (GLM, $\chi^2_1 = 3.5$, $P = 0.060$). A non-isotropic dispersal of aphids was observed whereas pots with dominance of *Ivo* (>50%) were not randomly distributed (experiment 5; fig. 1). *Ivo* was better represented than *Burk1* on high-populated plants.

Population growth in field cages

In the field, insecticide application had no significant effect on the density of aphids on both initially inoculated and non-inoculated plants but exerted a strong selection on the MLG composition by suppressing the population of *Ivo* (experiment 6; table 3). In the absence of insecticide, the proportion of *Ivo* was greater on inoculated plants than that on non-inoculated plants (GLM, $\chi^2_1 = 11.2$, $P < 0.001$), where *Burk1* was dominant (79%).

Identity of immigrant aphids

During the 2006 early cropping season, all *A. gossypii* alatae trapped within cotton fields presented the *Burk1* MLG (fig. 2). On cotton plants, *Burk1* represented 94% of the collected aphids, whereas *Burk8* (differing from *Burk1* only by one allele at locus Ago59) and *Ivo* represented respectively 4.5% and 1.5%. No *A. gossypii* alatae was trapped in corn fields. In the early off-season, *Burk1* and *Ivo* were poorly represented in traps placed within fields of okra and roselle as opposed to *C9*, which characterizes the *A. gossypii* race on Cucurbitaceae

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Table 3. Mean density of *Aphis gossypii* genotypes *Ivo* and *Burk1* and proportion of *Ivo* on inoculated and non-inoculated cotton plants (*G. hirsutum*) in field cages eight days after application of a mix of cypermethrin and profenofos, experiment 6 (95% confidence limits).

Treatment	Inoculated plants				Non-inoculated plants			
	Nb aphids per five leaves per plant		% *Ivo*		Nb aphids per five leaves per plant		% *Ivo*	
No insecticide	744	(420–1321)	58	(42–72)	92	(44–194)	21	(11–37)
Insecticide	1205	(768–1891)	5	(1–9)	183	(108–310)	0	
χ^2_3 (*P*)	1.7 (0.189)		27.6 (<0.001)		2.3 (0.132)		11.8 (<0.001)	

Nb, number.

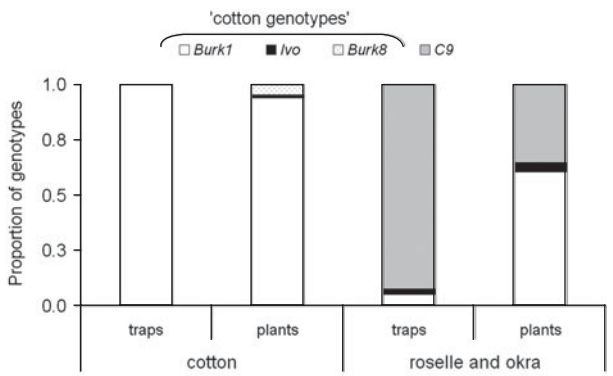

Fig. 2. Relative frequency of *Aphis gossypii* genotypes specialized on cotton *Burk1*, *Ivo* and *Burk8*, and of the genotype *C9* specialized on cucurbits, collected from traps and plants within the cotton cropping area of Cameroon (Djalingo, Mayo Dadi, Gaschiga and Pitoa) during 2006 early cropping season ($n=28$ and $n=67$, respectively) and early-off-season ($n=77$ and $n=71$, respectively).

(Carletto *et al.*, 2009). Nevertheless, *Burk1* was collected in significantly higher proportion than *Ivo* on okra and roselle ($\chi^2_1=51.45$, $P<0.001$).

Discussion

Competition and dispersal

When released concurrently with *Burk1* on the same plants, *Ivo* became the most abundant MLG, whereas no difference was observed when both clones were released independently on different plants, indicating that competition occurred. In field cages (experiment 6), the high density of *Burk1* on initially inoculated plants sprayed with insecticide (i.e. excluding *Ivo*), compared to unsprayed, initially inoculated plants indicates that competition with *Ivo* results in slowing down the population growth of *Burk1*. Fuller *et al.* (1999) proposed possible interclonal competition to explain a significant decrease in clonal diversity over a crop season in *A. gossypii* populations in commercial cucumber glasshouses. Competition between clones of *A. gossypii* was experimentally demonstrated in two Curcurbitaceae-specific genotypes (Rochat *et al.*, 1999). In field cage experiments (experiment 6), *Burk1* was better represented than *Ivo* on low-populated neighbouring plants, suggesting that dispersal may be a way to avoid competition on crowded plants. Similarly, when small-scale dispersal to neighbouring plants was possible in the laboratory (experiment 5), frequency of *Burk1* was >50% on some cotton plants where aphid density was low to moderate. This distribution pattern may have resulted from short-range dispersal behaviour in response to overcrowding (De Barro, 1992). In this experiment, we also observed a non-isotropic dispersal of aphids, possibly due to directional air flow created by the air conditioner.

The *A. gossypii* alatae trapped within or collected from newly planted cotton fields were all cotton-adapted genotypes but most presented the *Burk1* MLG. Cucurbitaceae-adapted genotypes (*C9*) were mainly collected from newly planted relay host plants at the beginning of the off-season (roselle and okra), but *Burk1* was again dominant among cotton-adapted genotypes. This prevalence of *Burk1* among cotton-adapted 'immigrants' may simply reflect a higher frequency within host plants from which they dispersed (source). Previous results showed that *Burk1* was highly prevalent on okra and roselle sampled during the 2003–2005 off-seasons in various locations in Cameroon (Brévault *et al.*, 2008). We also hypothesize that *Burk1* genotypes have higher dispersal ability than *Ivo* (experiment 6), possibly due to greater efficiency of host location (including flying ability and detection of host cues), greater quantity or earlier production of alatae. Long- and short-range dispersal by alate and apterous aphids, respectively, and subsequent colonization of new resources may be a way to avoid competition (Tilman, 1994; Friedenberg, 2003). Additional behavioural and demographic experiments could be considered to investigate such possibilities.

Selection by insecticides

Human-induced selective pressure, especially by widespread and repeated insecticide applications, probably shapes the genetic structure of *A. gossypii* populations feeding on cotton crops in favour of *Burk1* (Brévault *et al.*, 2008). A recent study showed multiple resistances to a broad range of insecticides and multiple mechanisms of resistance in *Burk1*, as opposed to *Ivo* (Carletto *et al.*, 2010). Insecticide resistance combined with clonal reproduction probably accounts for low genetic diversity and prevalence of the resistant genotype *Burk1* within and among field populations of the cotton host race. In contrast, persistence of the more susceptible genotype *Ivo* may be maintained by spatial and temporal heterogeneity of the agricultural landscape (Vorburger, 2006), including unsprayed host crops and wild plants from uncultivated habitats. Indeed, prevalence of *Ivo* was only observed in some populations collected from wild cotton plants (Ziguinchor, Senegal, 250 km away from the cotton-growing area) or untreated cotton plants from a collection (Maroua, Cameroon) planted during the off-season (Brévault *et al.*, 2008). Selection by insecticide treatments has also been proposed to explain the low genetic variability observed in the peach-potato aphid, *M. persicae*, in cultivated oilseed rape (Zamoum *et al.*, 2005).

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Moreover, a clonal turn-over due to fitness costs associated with insecticide resistance was observed in *M. persicae* populations in the absence of insecticide selection (Fenton *et al.*, 2005; Kasprowicz *et al.*, 2008a,b; van Toor *et al.*, 2008). In cotton aphids, the population of *Burk1* was smaller than that of *Ivo* when both genotypes were concurrently transferred on the same plants (competition), whether on cotton or relay host plants, suggesting potential pleiotropic effects conferred by resistance mutations which could affect the ability of insecticide-resistant aphids to respond to over-crowding conditions. For example, higher carboxylesterase levels in the peach-potato aphid appear to be closely associated with potential maladaptive behaviour in the form of lower tendencies to move between plants and from deteriorating leaves in particular (Foster *et al.*, 2002). Natural enemies (pathogens, parasitoids and predators) are also selective factors that could affect the clonal diversity of aphid asexual populations, as demonstrated among genotypes of *A. pisum* Harris (Henter & Via, 1995; Ferrari *et al.*, 2001) and *M. persicae* (von Burg *et al.*, 2008). In *M. persicae*, insecticide-resistant genotypes carrying different combinations of a *kdr* mutation and extreme carboxylesterase resistance show a reduced response to aphid alarm pheromones (Foster *et al.*, 2005, 2007), which in turn may affect their ability to respond to predation and parasitism. Regarding cotton-adapted genotypes of *A. gossypii* in WCA, additional data are needed to understand how possible fitness costs could affect the ability of insecticide-resistant genotypes to respond to interclonal competition. In particular, demographic and behavioural studies of *Burk1* and *Ivo* genotypes, including life history (longevity, fecundity, development time, production of winged morphs, etc.), interactions between individuals, dispersal ability, habitat selection and response to natural enemies, could provide relevant information. Climatic conditions typical of the dry season, i.e. high temperature and low relative humidity, had no significant effect on the distribution of the two genotypes on cotton plants compared to the conditions of lower temperature and higher humidity in the growth chamber, with *Ivo* again dominating *Burk1*. Nevertheless, genetic variation among clones in their temperature tolerance has been detected in other aphid species (Griffiths & Wratten, 1979; Vorburger, 2004).

In WCA, cotton pest management has been largely based on the use of broad-range insecticides, mainly organophosphates and pyrethroids. Furthermore, these insecticides have been concurrently sprayed by smallholders on vegetable crops which act as relay host plants for cotton-adapted genotypes of *A. gossypii*. This time and area-wide selection pressure has probably led to the prevalence of a unique and multi-resistant genotype, *Burk1*, which threatens the sustainability of cotton-based cropping systems, both from an ecological and an economic perspective. In those agricultural landscapes, both cotton fields and relay host plants serve as sources for *Burk1* and as sinks for *Ivo*. Accordingly, it would be of great interest to assess whether reduction in the use of broad-range insecticides to control cotton bollworms (e.g. integrated pest management) could restore genetic diversity in cotton-adapted aphids. Based on on-farm experiments, Achaleke *et al.* (2009) confirmed the suitability of selective insecticides such as spinosad and emamectin-benzoate for the control of bollworms. Also, the use of IPM-compatible insecticides, such as neonicotinoids (acetamiprid) or flonicamid, should be encouraged on vegetable crops to control aphids. As pointed out by Carletto *et al.* (2010), *Burk1* is susceptible to acetamiprid.

The adoption of genetically engineered cotton that expresses *Bacillus thuringiensis* (*Bt*) toxins that do not control sap-sucking pests (Showalter *et al.*, 2009) could also impact the genetic diversity of cotton-adapted aphids. Moreover, it has been shown that aphid density in *Bt* cotton crops did not increase dramatically compared to conventional cotton plots treated with insecticides for the control of *Helicoverpa armigera* (Hübner), probably because aphids natural enemies were preserved (Wu & Guo, 2003). In WCA, high rates of *A. gossypii* parasitism by *Aphelinus albipodus* (Hayat and Fatima) (Hymenoptera: Aphelinidae) have been observed at the end of the cropping season when insecticides are no longer sprayed on cotton fields (S. Nibouche, unpublished data). On the other hand, a bivalent transgenic cotton expressing both a *Bt* endotoxin gene and a protease inhibitor gene (a cowpea trypsin inhibitor gene) was reported to negatively affect survival, fecundity, longevity and feeding behaviour of aphid in the first two generations, but aphid fitness soon increased in the third generation (Liu *et al.*, 2005). It was suggested that this adaptation to trypsin inhibitors resulted from phenotypic plasticity in clones across parthenogenetic generations. However, it would be interesting to demonstrate that there was no genetic variability between the clones used in this experiment that could be selected for to overcome trypsin inhibitors expressed in transgenic cotton.

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