

1 **Journal**

2 International Journal of Pest Management

3 **Article Type**

4 Original Research Papers

5 **Title**

6 Relationship of phenotypic structures and allelochemical compounds of okra (*Abelmoschus*
7 spp.) to resistance against *Aphis gossypii* Glover

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28 **Relationship of phenotypic structures and allelochemical compounds of okra**
29 **(*Abelmoschus* spp.) to resistance against *Aphis gossypii* Glover**

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39 The cotton aphid (*Aphis gossypii*) is one of the major pests of okra. Eleven okra
40 varieties collected from farmers' fields in Cameroon and four aphid-resistant
41 accessions from AVRDC were evaluated from October 2012 to March 2013, and
42 another seven aphid-resistant accessions from AVRDC were evaluated from March to
43 July 2013 at AVRDC's Nkolbisson station, Yaoundé, Cameroon. Accessions selected
44 from these trials were screened in an advanced confirmatory screening in autumn
45 (October to December) 2013 in Shanhua, Taiwan. Results showed that none of the
46 farmers' landraces were resistant to aphids. Two accessions (VI041210 and
47 VI033824) with the lowest aphid populations had the highest trichome density. Among
48 the phytochemicals analysed, higher nitrogen and potassium levels made the okra
49 accessions susceptible to aphids during the reproductive stage of the plants. Similarly,
50 the total phenol content was lower in moderately resistant accessions during their
51 reproductive stage. Aphid feeding induced an increase in phenols, and to a lesser
52 extent, tannins. Accessions VI041210 and VI033824 could be incorporated into an
53 integrated pest management strategy to enhance productivity of okra.

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55 **Keywords:** resistance, okra, aphids, biophysical and biochemical bases, feeding
56 induced responses.

57 **1. Introduction**

58 Okra belongs to the *Abelmoschus* genus in the family Malvaceae. It was formerly included in
59 the genus *Hibiscus*. Cultivated species are West African okra [*Abelmoschus caillei* (A. Chev.)
60 Stevels], common okra [*A. esculentus* (L.) Moench.], aibika [*A. manihot* (L.) Medik.] and a
61 fourth domesticated species *A. moschatus*. *A. manihot* usually does not flower and it is
62 extensively cultivated for its leaves in Papua New Guinea, Solomon Islands and other South
63 Pacific Islands (Kumar *et al.*, 2010). *A. moschatus* is cultivated for culinary as well as
64 medicinal purposes in India, South Togo and Benin (Hamon and Sloten, 1995). Okra (*A.*
65 *esculentus* and *A. caillei*) is an important vegetable in South- and Southeast Asia and sub-
66 Saharan Africa. Okra is produced in an area of over 1 million hectare worldwide, with Asia
67 and Africa having about 0.5 Mha, each (FAO, 2012). India is the world's leading okra
68 producer (6 million t annually), followed by Nigeria (1.1 million t). Cameroon produces about
69 64,000 t annually in an area of about 26,500 ha. Asia and Africa have almost similar acreage
70 under okra production, but the average productivity in Africa is very low (3.55 t/ha) compared
71 to Asia (11.52 t/ha). Although several factors contribute to the low productivity of okra in
72 Africa, biotic constraints are a major limiting factor.

73 The cotton or melon aphid, *Aphis gossypii* Glover (Homoptera: Aphididae) is one of the major
74 constraints to okra production in Cameroon. *A. gossypii* is found in tropical and temperate
75 regions of the world, except in extreme northern areas. It has a wide range of almost 700
76 host plants worldwide (Capinera, 2000). Besides causing direct damage, *A. gossypii* is
77 known to transmit more than 50 plant viruses (Blackman and Eastop, 2007). The cotton
78 aphid usually infests the underside of the okra leaves. In severe infestations, the pest is also
79 found on the stems, fruits and upper leaf surface. Aphids cause damage either directly by

80 feeding, which results in curling and deformation of young leaves and stems, or indirectly by
81 contaminating the leaves and fruits with honeydew, which favours the growth of black sooty
82 mould that inhibits photosynthesis, thus causing substantial yield loss (Capinera, 2000).
83 Honeydew attracts ants that fend off natural enemies of aphids (Yokomi and Tang, 1995).
84 Chemical treatment remains the main method of control, although resistance to insecticides
85 has been documented in many *A. gossypii* populations (Owusu and Yeboah, 2007). Hence,
86 alternative pest management strategies are needed.

87 Plant resistance is an important component in integrated pest management (IPM) strategies
88 against aphids. Plant morphological characters play a vital role in imparting resistance to
89 various insect pests. Although we do not find many references on the importance of plant
90 characteristics in okra offering resistance to *A. gossypii*, evidences are available in cotton,
91 another Malvaceous species. Trichomes in cotton and okra have either adverse (Zarpas *et al.*,
92 2006; Abang *et al.*, 2014) or positive effects (Nibouche *et al.*, 2008) on resistance to *A.*
93 *gossypii*. Morphological or structural characteristics such as silica content, leaf toughness,
94 deceptive plant structures and leaf size in cotton also enhance plant resistance (Deguine and
95 Hau, 2001). These morphological characters influence aphids' settling and feeding behaviour.
96 For instance, after 72 h of infestation, most of the *A. gossypii* left the leaves of resistant
97 melon plants, since they found them unsuitable for feeding and colonization (Soria *et al.*,
98 2000). However, we did not find any such preference or avoidance behavior on resistant and
99 susceptible okra accessions in our earlier study (Abang *et al.*, 2014). On an aphid transmitted
100 virus resistant melon plant, *A. gossypii* seldom reached the phloem, or stopped feeding when
101 phloem was reached (Klingler *et al.*, 1998), and then starved.

102 Plants produce biochemicals that are involved in plant defence. For instance, terpenoids are
103 involved in defence as toxins and feeding deterrents; they are also common in plants, and
104 are used as active ingredients of pesticides (Turlings *et al.*, 1995). Phenols, most commonly
105 tannins, act as toxins and repellents, and also bind to insect salivary proteins. Ma *et al.* (2005)
106 reported significant feeding inhibitions to *A. gossypii* on artificial diet containing tannic acid,

107 since it reduced the total probing time. Secondary metabolites containing nitrogen and
108 sulphur, such as alkaloids and non-protein amino acids, act as protein inhibitors, deactivators,
109 toxic substances, and irritants. Plants also produce volatile organic compounds, especially
110 during induced resistance, to repel herbivores and attract natural enemies (Dicke and Van
111 Loon, 2000; De Moraes *et al.*, 2001; Kessler and Baldwin, 2001). Hegde *et al.* (2012)
112 confirmed that antixenosis in cotton was activated by the natural plant defense elicitor cis-
113 jasmone in the presence of *A. gossypii*. The levels of free amino acids and sugars in cotton
114 may partially determine the likelihood of *A. gossypii* infestation (Deguine and Hau, 2001).
115 Plant nitrogen content is an indicator of food quality and host selection by *A. gossypii*, since
116 amino-acids are important for the development of *A. gossypii* (Slosser *et al.*, 1989).

117 Since host plant resistance to *A. gossypii* based on bio-physical and bio-chemical
118 characteristics in Malvaceous species such as cotton has been confirmed, we aimed to
119 screen the okra germplasm for their resistance to *A. gossypii*. Hence, the objectives of this
120 study were (i) to identify the *Abelmoschus* spp. genotypes that are resistant to *A. gossypii* in
121 Cameroon, and (ii) to characterize the biophysical and biochemical bases of aphid resistance
122 that lead to antixenosis and antibiosis.

123

124 **2. Materials and methods**

125 **2.1. Field screening of okra germplasm against aphids**

126 Our earlier study confirmed the genetic similarity of *A. gossypii* populations in Taiwan and
127 Cameroon based on the mitochondrial cytochrome c oxidase I (*coxI*) phylogenetic analysis
128 (Abang *et al.*, 2014). Hence, shuttle screening of the okra germplasm was carried out in
129 Taiwan as well as Cameroon to expedite the identification of aphid resistant okra accessions.
130 Eleven okra varieties collected from farmers' fields in Cameroon, and four accessions rated
131 as aphid-resistant from a non-replicated preliminary screening trial in Taiwan that involved a
132 total of 107 accessions were evaluated in a replicated trial from October 2012 to March 2013.
133 A known susceptible accession (VI057245) also was included. Subsequently, seven

134 accessions rated as aphid-resistant from another non-replicated preliminary screening trial
135 involving 64 accessions in Taiwan were evaluated in the second replicated trial from March
136 to July 2013. These two trials were conducted in Nkolbisson, Yaoundé, Cameroon
137 (03°51.79'N and 11°27.71'E). Accessions selected from these two trials including the
138 susceptible accession were screened in an advanced confirmatory trial in October 2013 to
139 January 2014 in Shanhua, Taiwan. The seeds were sown on October 5th and a plant to plant
140 spacing of 1-m, and a row to row spacing of 1.5-m were maintained. These trials were
141 conducted using a randomized block design with three replications. The trials were
142 maintained following customary cultural practices, and without pesticide application to control
143 aphids or other sucking insects to increase the natural aphid infestation. Aphid populations
144 were directly scored at weekly intervals starting from four weeks after transplanting in the
145 field. The aphid population was scored on three leaves (one each from the bottom, middle
146 and top) of ten randomly selected plants in each replication, using the following rating scale:
147 0 = no aphids present; 1 = 1 to 10 aphids per leaf; 2 = 11 to 100 aphids per leaf; 3 = 101 to
148 1000 aphids per leaf; and 4 = >1000 aphids per leaf. When the aphids were less than 100,
149 they were directly counted on selected leaves. However, the aphids were collected from
150 selected leaves and counted, when the colonies had high number of aphids. The scored data
151 from each accession was expressed as the area under the infestation pressure curve
152 (AUIPC), and calculated using the following formula modified from Shaner and Finney (1977):

$$\sum_{i=1}^{n-1} \frac{(Y_i + Y_{i+1})}{2} (t_{i+1} - t_i)$$

153

154 Where n= number of assessment times, and Y = number of insects at time t.

155

156 **2.2. Biophysical and biochemical bases of resistance**

157 **2.2.1. Biophysical bases of resistance**

158 The biophysical bases of resistance, *viz.*, leaf trichome density (Bourland *et al.*, 2003) and
159 toughness were examined in selected resistant and susceptible accessions (VI033824,

160 VI039614, VI041210, VI060688, VI060794, VI060818, and VI057245) (Abang *et al.*, 2014).
161 Three leaves, one each from the bottom, middle and top of the plant were collected at the
162 beginning of flowering. For leaf trichome density, 1 cm² leaf pieces were collected from either
163 side of the leaf mid-vein. The leaf pieces were mounted on a stereomicroscope and the
164 number of trichomes was counted. For measuring the leaf toughness, 1 cm² leaf pieces were
165 collected from either side of the mid-vein and mounted on a gram gauge. A 0.52-mm
166 diameter blunt probe was used to measure the force required to puncture leaf tissues. The
167 gram gauge was designed by modifying a scale balance using a method described by
168 Wheeler and Center (1997). Measurements of leaf toughness and trichome densities were
169 recorded in five replicates with two plants per replicate for each accession.

170

171 **2.2.2. Biochemical bases of resistance**

172 Tannins, total sugar, reducing sugars, phenolic compounds, total nitrogen and potassium
173 were estimated in okra leaves of selected resistant and susceptible accessions (Abang *et al.*,
174 2014). The plants were grown in a controlled environment with aphid infestation (for induced
175 response) and without aphid infestation (for constitutive resistance), following the completely
176 randomized design (CRD). Two separate plants were used at 6 and 10 weeks after sowing
177 (vegetative and reproductive stage) (Shannag, 2007). To study induced response, 10 aphids
178 at 6 weeks, and 25 aphids at 10 weeks were released on each plant to feed and reproduce
179 for 5–6 days following the protocol of Messina and Bloxham (2004). All aphids on infested
180 plants were counted to estimate aphid density at the end of the infestation. Six leaves from
181 infested and uninfested plants (two each from the top, middle and bottom of the plant) were
182 collected and dried at -56 °C for 7 days in a freeze dryer. The dry samples were ground in an
183 electric blender. The samples were replicated five times for each accession, with two plants
184 per replication.

185 Analysis of tannins in leaves of aphid-resistant and susceptible okra accessions was
186 conducted using the Catechin standard and acidified vanillin method (Broadhurst and Jones,

187 1978). One gram of powder sample per replication was used for analysis. Total sugar content
188 of leaves in aphid-resistant and susceptible okra accessions was spectrophotometrically
189 determined using Anthrone reagent (Dreywood, 1946). Anthrone reagent reacts specifically
190 with carbohydrates in a concentrated sulphuric acid solution to produce a blue-green colour
191 at 630 nm. The results were expressed as sucrose equivalents. Reducing sugar content was
192 determined using the Nelson-Somogyi Method (Somogyi, 1952). Reducing sugars, when
193 heated with alkaline copper tartrate, reduce the copper from the cupric to cuprous state and
194 thus cuprous oxide is formed. When cuprous oxide is treated with arsenomolybdic acid, the
195 reduction of molybdic acid to molybdenum blue takes place. The blue colour developed is
196 compared with a set of standards in a colorimeter at 620 nm. The colorimetric method of
197 Folin-Denis as described by Swain and Hillis (1959) was used to determine the phenolic
198 compounds in the leaves. Total leaf nitrogen was determined following Kjeldahl's method as
199 described by Bremner and Mulvaney (1982). The Potassium content in the digested solution
200 of plant tissues was diluted and analyzed by flame-photometer as described by Rayment and
201 Lyons (2011).

202 **2.3. Antixenosis**

203 Antixenosis or non-preference was investigated by studying the settling behaviour of aphids.
204 Plants were infested with ten aphids, after two hours of starvation, on the upper surface of
205 the second fully expanded leaf from the apex. After 72 h, adult aphids were counted to
206 evaluate their permanence on the infested leaf, and leaves with four or less aphids were
207 considered non-preferred by aphids (Ferreles, 1994).

208

209 **2.4. Statistical analysis**

210 The AUIPC values for aphid population per leaf were subjected to a statistical analysis based
211 on mean (m) and standard deviation (sd) and categorized as follows: the accessions that had
212 mean damage score (n) less than $m-2sd$ were considered highly resistant; between $m-1sd$ to
213 $m-2sd$ as resistant; between m to $m-1sd$ as moderately resistant; between m to $m+1sd$ as

214 moderately susceptible; between m+1sd to m+2sd as susceptible; and more than m+2sd as
215 highly susceptible.

216

217 Data obtained from experiments on the biochemical and biophysical traits of selected okra
218 accessions were subjected to analysis of variance (ANOVA) with the Proc GLM procedure of
219 SAS, version 9.1 (SAS Institute, Cary, NC, U.S.A.). The choice test on the settling behaviour
220 of aphids was analysed using the Kruskal-Wallis test with one-way ANOVA and non-
221 parametric test. Where significant differences were found between treatments, Tukey's
222 honestly significant difference (HSD) test was used as a *post hoc* procedure for comparison.

223

224 **3. Results**

225 **3.1. *Field screening of okra germplasm against aphids***

226 Out of the 16 varieties/accessions screened in the first trial (October 2012–March 2013), only
227 one accession (VI033824) was rated as resistant, whereas ten accessions/varieties were
228 moderately resistant (Table 1). None of the farmers' varieties were resistant. In the second
229 trial (March–July 2013), out of seven accessions, five were moderately resistant and none
230 were resistant. Results of the advanced confirmatory screening trial during November 2013 –
231 January 2014 showed that four accessions (VI041210, VI057245, VI033824 and VI060818)
232 including the susceptible accession were moderately resistant (Table 1).

233

234 **3.2. *Biophysical and biochemical bases of resistant accessions***

235 **3.2.1. *Biophysical bases***

236 There were significant differences in leaf trichome density in leaves from top ($P < 0.0001$),
237 middle ($P = 0.005$) and bottom ($P = 0.0004$) strata among the accessions. In general, the
238 trichome density was significantly higher in the top three moderately resistant accessions
239 (VI041210, VI057245 and VI033824) than in the other accessions (Table 2). The trichome
240 density was significantly lower in the susceptible accession (VI060794). For leaf toughness,

241 there were no significant differences among the accessions for the force needed to puncture
242 the leaves from top ($P=0.41$), middle ($P=0.11$) and bottom ($P=0.14$) strata (Table 2).

243

244 **3.2.2. Biochemical bases**

245 Biochemical studies of selected okra accessions at six weeks after sowing showed that there
246 were significant differences between accessions in total phenols ($P=0.022$) and total tannins
247 ($P=0.017$) of plants without aphids, but not in plants with aphids. Total phenols were higher in
248 plants infested with aphids than in plants without aphids; the opposite was observed for total
249 tannins (Table 3). Although one of the top ranking moderately resistant accessions
250 (VI057245) had higher total phenol, it was not significantly higher than the susceptible
251 accession VI060794. The other two top ranking moderately resistant accessions (VI041210
252 and VI033824) had less total phenol, but were comparable with the susceptible accession
253 (VI060794). Hence, the total phenol content among the accessions did not reveal any clear
254 relationship with aphid resistance. When the plants were not infested with aphids, one of the
255 top ranking moderately resistant accessions (VI057245) had significantly lower tannins than
256 the susceptible accession VI060794. However, the tannin content among the other
257 accessions did not reveal any clear relationship with aphid resistance. Total sugars showed
258 no significant differences between accessions infested with or without aphids. However,
259 when infested with aphids, the reducing sugar content was higher in one of the moderately
260 resistant accessions (VI057245). There were significant differences in total nitrogen among
261 the selected okra accessions, infested with aphids ($P=0.0006$), and two moderately resistant
262 accessions (VI057245 and VI041210) had significantly lower nitrogen content than the
263 susceptible accession. However, potassium content did not show any relationship with aphid
264 resistance in selected okra accessions.

265 At 10 weeks after sowing, there were significant differences in total phenols among the okra
266 accessions, only for plants infested with aphids ($P=0.036$) (Table 4). Unlike the younger
267 plants, okra accessions at this stage clearly showed the difference in total phenols among

268 the resistant and susceptible accessions. For instance, total phenols were significantly lower
269 in three moderately resistant accessions (VI041210, VI057245 and VI033824) than in the
270 susceptible accession (VI060794). For tannins, significant differences were found between
271 the accessions in both plants infested with ($P=0.009$) or without ($P=0.007$) aphids. However,
272 a clear difference in tannins among the susceptible and resistant accessions did not exist,
273 except in VI041210 (plants without aphids), which had significantly lower levels of tannins
274 than the susceptible accession. Total sugars also showed significant differences between
275 accessions with ($P=0.025$) and without ($P=0.0003$) aphids, but only VI033824 (without aphid
276 infestation) had significantly higher total sugars than the susceptible accession. Total
277 nitrogen showed significant difference between accessions without aphids ($P<0.0001$) only.
278 The susceptible accession (VI060794) had significantly higher total nitrogen than other
279 accessions. Similarly, potassium content in the susceptible accession was also higher than
280 the moderately resistant accessions, in both with or without aphid infestation.

281 **3.3. Antixenosis**

282 The results indicated that moderately resistant accessions did not induce antixenosis. There
283 were no significant differences between the accessions in number of aphids per leaf after 72
284 h of release ($P=0.6861$). Thus, aphid number did not differ on susceptible and resistant okra
285 accessions (Table 5).

286

287 **4. Discussion**

288 This study included several popular okra varieties collected from farmers' fields in Cameroon.
289 However, none of these varieties were resistant to *A. gossypii*. This indicates that the pest
290 status of *A. gossypii* on okra is high in Cameroon because of the lack of resistant varieties.
291 Out of the seven accessions screened in the confirmation trials, four accessions (VI041210,
292 VI057245, VI060818 and VI033824) were found to be moderately resistant. Although
293 VI057245 was included as the known susceptible accession, it was consistently moderately
294 resistant in both the trials in Cameroon and the final trial in Taiwan. This suggests that

295 resistance to aphids may vary in space and time. Besides, the resistance may also vary
296 depending on the host race, because *A. gossypii* is structured into geographically
297 widespread host races specialized on Cucurbitaceae, cotton, eggplant or pepper (Carletto *et*
298 *al.*, 2010). We used the race from okra, which may be similar to cotton because they belong
299 to Malvaceae. However, the selected okra accessions could display different reactions to
300 non-cotton or okra races of *A. gossypii*, if they are present in a location where the selected
301 resistant accessions are screened. Hence, repeated screening trials should be conducted
302 before discarding the germplasm materials. VI041210 was an old cultivar, collected at
303 Cabaroan (17°39' N, 120°22' E), Santa Catalina, Ilocos Sur province in the Philippines.
304 VI057245 was a landrace collected at Cham Ko Louk (13°26'56.1" N, 103°00'13.5"), Banteay
305 Meanchey province in Cambodia. VI060818 was a local cultivar (ORS 354) from Mali,
306 whereas VI033824 was an old cultivar collected at Koronadal, Barrio (06°30' N, 124°50' E) in
307 South Cotabato province of the Philippines. All these accessions were the Asian okra
308 species *A. esculentus*. Our earlier study revealed that *A. esculentus* accessions originating
309 from the Philippines possess appreciable levels of resistance to *A. gossypii* (Abang *et al.*,
310 2014). However, it is important to evaluate their adaptation to Cameroon conditions in future
311 studies to confirm their yield potential before recommending them for farmers' use.

312 Although West African okra (*A. caillei*) accounts for only 5% of the total world okra production
313 (Siemonsma and Kouame, 2004), it is a very important crop in the major okra producing
314 countries in Africa. However, it has to be noted that the aphid resistant accessions identified
315 in the current study were only the Asian okra species *A. esculentus*. Kumar *et al.* (2010)
316 suggested that earliness of *A. esculentus* as compared to *A. caillei* was preferred in the
317 Sudano-Sahel. Hence, these aphid resistant accessions could be directly cultivated in these
318 regions after local validation. However, the adaptability of these *A. esculentus* accessions
319 should be confirmed before promotion, because *A. caillei* has gradually replaced *A.*
320 *esculentus* in the tropical-humid region because of its better adaptation under humid zone
321 (Siemonsma, 1982). Alternatively, aphid resistant *A. esculentus* accessions can be used in

322 resistance breeding programs, since inter-specific cross between *A. caillei* and *A. esculentus*
323 is successful with the possibility of gene transfer, although the partial hybrid breakdown
324 barrier must be overcome (Fatokun, 1987). However, the use of okra accessions identified in
325 this study as parental lines in breeding programs may not be advisable, since the aphid
326 resistance is only moderate.

327 The biophysical traits of the plants play an important role in the colonization, feeding and
328 development of *A. gossypii* on okra. Trichomes in cotton and okra have either adverse
329 (Zarpas *et al.*, 2006; Abang *et al.*, 2014) or positive effects (Nibouche *et al.*, 2008) on
330 resistance to *A. gossypii*. In the present study, the biophysical bases (such as leaf trichomes)
331 were significantly different between resistant and susceptible accessions. Three top-ranking
332 moderately resistant accessions that had lower AUIPC values had higher trichome densities.
333 This suggests that leaf trichomes are one of the factors that influence the colonization of *A.*
334 *gossypii* on okra plants. Similar observations were made in our earlier study (Abang *et al.*,
335 2014) as well as by Santos *et al.* (2003) and Soglia *et al.* (2002 & 2003). Although both
336 glandular and non-glandular types of trichomes have been described in the genera *Sida* and
337 *Hibiscus* of Malvaceae (Shaheen *et al.*, 2009a&b), only three non-glandular trichome types
338 (unicellular filiform hairs, unicellular conical hairs and stellate hairs) have been described in
339 *Abelmoschus* (Osawaru *et al.*, 2011). Since we did not study the types of trichomes in these
340 selected moderately resistant accessions, detailed studies may be carried out in the future, if
341 one wants to exploit these accessions. Some studies showed that *A. gossypii* populations
342 prefer young and succulent leaves, which are generally located on the apical parts of
343 susceptible varieties (Santos *et al.*, 2003; Chau *et al.*, 2005). Non-occurrence of aphids on
344 the apical leaves of resistant varieties is an indication that these leaves have more trichomes,
345 which can make locomotion, feeding and reproduction of *A. gossypii* difficult (Soglia *et al.*,
346 2002, 2003; Santos *et al.*, 2003). In the current study also, significantly higher number of
347 trichomes were found in the leaves from the top stratum among the three studied strata.
348 Hence, trichome density in the top strata of the plants may be considered while selecting the

349 accessions for their resistance to *A. gossypii* in the future. In addition to trichomes, leaf
350 hardness is important in enhancing plant resistance (Deguine and Hau, 2001). However, our
351 current study did not find any evidence supporting the influence of leaf hardness against
352 aphid infestation on resistant or susceptible accessions.

353 Plant chemistry also affects pest infestations. Biochemical studies of selected okra
354 accessions at 6 and 10 weeks after sowing showed that total phenols were higher in plants
355 infested with aphids than in plants without aphids in most accessions. It has been reported
356 that some of the traits and processes that defend plants against pests change following pest
357 attacks (Khattab, 2007; Wilson *et al.*, 2011). The susceptible accession had higher total
358 phenols than the moderately resistant accessions during the reproductive stage of okra.
359 Zucker (1982) found an inverse correlation for the effect of total phenols in *Populus*
360 *angustifolia* to the galling aphid, *Pemphigus betae*. However, our results are contrary--total
361 phenol content is positively correlated with the aphid infestation. This was not the case in our
362 earlier study, in which the leaf total phenols did not play a significant role in imparting
363 resistance in okra to aphids (Abang *et al.*, 2014). In addition, when there was an aphid
364 infestation, the plant defence compounds, especially phenolic compounds, were increased. A
365 similar rapid increase in total phenols following insect damage was recorded by Tuomi *et al.*
366 (1988). As documented in our previous study (Abang *et al.*, 2014), our current study also
367 confirmed that tannins did not have any significant role in imparting resistance to *A. gossypii*
368 in okra accessions, although Ma *et al.* (2005) found that tannins affected *A. gossypii* feeding.
369 Total sugars and reducing sugars did not have a significant role in offering resistance in
370 plants infested with or without aphids at all growth stages. However, total nitrogen at all
371 growth stages and potassium at the reproductive stage of okra were higher in the susceptible
372 accession. Although Leite *et al.* (2007) confirmed that leaf nitrogen and organic compounds
373 in okra did not have any effects on *A. gossypii* populations, and Lu *et al.* (2009) found that
374 total nitrogen and amino acids in cotton were not associated with resistance to *A. gossypii*,

375 the current study revealed that the total nitrogen increased the susceptibility of okra
376 accessions to aphids.

377 The number of aphids did not differ between the okra accessions in the antixenosis study.
378 However, leaf trichome density was lower, and nitrogen and potassium content were higher
379 in leaves of susceptible VI060794 than in the moderately resistant accessions. These
380 parameters did not affect aphid settling behaviour, but may be responsible for subsequent
381 population build-up on the susceptible accession. It should also be noted that VI060794 was
382 not a highly susceptible accession, and it showed a moderate resistance in a trial in
383 Cameroon during March-July 2013. Since this was a newly initiated aphid resistance
384 screening program involving the okra germplasm from AVRDC – The World Vegetable
385 Center, and we did not have a well-known aphid susceptible okra line, we chose one of the
386 susceptible accessions from the available list. Hence, some of the results from bio-chemical
387 studies differ from earlier studies on other crops or in-conclusive, and hence need further
388 confirmation. Future studies on antibiosis will certainly help explain the difference in aphid
389 infestation levels.

390 **5. Conclusions**

391 Four okra accessions (VI041210, VI057245, VI060818 and VI033824) were identified as
392 moderately resistant to aphids. Leaf trichome density especially on leaves from top stratum
393 plays a major role in okra resistance to aphids. Two accessions (VI041210 and VI033824)
394 with the lowest aphid population, highest trichome density, and lower leaf nitrogen content
395 are potential sources of resistance to *A. gossypii* in okra. The primary metabolites and plant
396 nutrients did not change with aphid infestation, except secondary metabolites such as
397 phenols and tannins. It seems that aphid feeding induced a response not related to okra
398 primary metabolites and minerals, although it needs further confirmation. The effects of plant
399 parameters did not affect aphid settling behaviour. Accessions VI041210 and VI033824 could
400 be incorporated into integrated pest management strategies to enhance productivity,
401 incomes and livelihoods of okra farmers.

402 **Acknowledgements**

403 We thank the Federal Ministry for Economic Cooperation and Development, Germany for
404 financing this study, and the collaboration of the University of Yaoundé I, Cameroon.

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525

526 **Table captions**

527 Table 1. Area under the infestation pressure curve (AUIPC) of okra accessions assessed for
528 aphid numbers from October 2012 to January 2014.

529 Table 2. Leaf hairiness and hardness in selected okra accessions

530 Table 3. Biochemical content of okra accessions with and without aphids at vegetative
531 growth (6 weeks after sowing)

532 Table 4. Biochemical content of okra accessions with and without aphids at reproductive
533 growth (10 weeks after sowing)

534 Table 5. Settling behavior of aphids on selected okra accessions