REPUBLIQUE DU CAMEROUN
Paix – Travail – Patrie
****
UNIVERSITE DE YAOUNDE I
FACULTE DES SCIENCES
DEPARTEMENT DE DE BIOLOGIE ET PHYSIOLOGIE ANIMALES
****

REPUBLIC OF CAMEROUN
Peace – Work – Fatherland
****
UNIVERSITY OF YAOUNDE I
FACULTY OF SCIENCE
DEPARTMENT OF OF ANIMAL BIOLOGY AND PHYSIOLOGY
****

Interactions between the germplasm of okra (Abelmoschus Spp) and Aphids with special reference to Aphis gossypii Glover (Hemipterz: Aphididae) in Cameroon

THESIS
Submitted in partial fulfilment of the requirements for the award of a Doctorat/Ph.D Degree in Animal Biology

Par : **ABANG Albert FOMUMBOD**
Master of Science in Animal Biology

Sous la direction de
KEKEUNOU Sévilor
Associate professor
RAMASAMY Srinivasan
Senior Scientist

Année Académique : 2018

UNIVERSITE DE YAOUNDE I
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FACULTE DES SCIENCES
THE FACULTY OF SCIENCE

CERTIFICATE OF CORRECTION

We, the undersigned, members of jury for the defense of Doctorate/PhD thesis of animal biology and physiology (option: zoology), of M. ABANG ALBERT FOMUMBOD, Registration number: 08Q1250, defense authorized by correspondence N° 7_1770/UYI/VREPDTIC/DAAC/DEPE/CB-nsr of the Vice Chancellor of the University of Yaoundé I, on December 22, 2017, certify that the corrections required of the candidate during this evaluation carried out on February 26, 2018 have been made, and that this document may be deposited in its current form.

In witness whereof the present testimonial is given with the privileges thereunto pertaining / -

Done in Yaoundé on: ...08 MAY 2018.....

The examiners

The President of the jury

The head of Department

Pr Charles Félix
NJILONG BILONG
PROFESSEUR

UNIVERSITE DE YAOUNDE I Faculté des Sciences Division de la Programmation et du Suivi des Activités Académiques		THE UNIVERSITY OF YAOUNDÉ I Faculty of Science Division of Programming and Follow-up of Academic Affaires
LISTE DES ENSEIGNANTS PERMANENTS		LIST OF PERMENENT TEACHING STAFF

SCHOOL YEAR 2017/2018

(By Department and by Grade)

DATE UPDATED: (10 January 2018)

ADMINISTRATION

DEAN: AWONO ONANA Charles, Professor

VICE-DEAN / DPSAA : DONGO Etienne, Professor

VICE-DEAN / DSSE : OBEN Julius ENYONG, Professor

VICE-DEAN / DRC : MBAZE MEVA'A Luc Léonard, Associate Professor

Head of Administrative and Financial Division : NDOYE FOE Marie C. F., Associate Professor

Head of Division of Education, Research and Academic Affaires: ABOSSOLO Monique, Associate Professor

1- DEPARTMENT OF BIOCHEMISTRY (BC) (41)			
N°	SURNAME AND GIVEN NAMES	GRADE	OBSERVATIONS
1	FEKAM BOYOM Fabrice	Professor	In service
2	MBACHAM FON Wilfried	Professor	In service
3	MOUNDIPA FEWOU Paul	Professor	Head of Department
4	BENG née NINTCHOM PENLAP V. épse BENG	Professor	In service
16	OBEN Julius ENYONG	Professor	*Vice-Dean (DSSE)*
5	ATOGHO Barbara Mma	Associate Professor	In service

6	BELINGA née NDOYE FOE M. C. F.	Associate Professor	Head DAF / FS
7	BIGOGA DIAGA Jude	Associate Professor	In service
8	BOUDJEKO Thaddée	Associate Professor	In service
9	EFFA NNOMO Pierre	Associate Professor	In service
10	FOKOU Elie	Associate Professor	In service
11	KANSCI Germain	Associate Professor	In service
12	NANA Louise epouse WAKAM	Associate Professor	In service
13	NGONDI Judith Laure	Associate Professor	In service
14	NGUEFACK Julienne	Associate Professor	In service
15	NJAYOU Frédéric Nico	Associate Professor	In service
17	ACHU Merci BIH	Lecturer	In service
18	BIYITI BI ESSAM née AKAM ADA L.	Lecturer	CT MINRESI
19	DEMMANO Gustave	Lecturer	In service
20	DJOKAM TAMO Rosine	Lecturer	In service
21	DJUIDJE NGOUNOUE Marcelline	Lecturer	In service
22	DJUIKWO NKONGA Ruth Viviane	Lecturer	In service
23	EVEHE BEBANDOUE Marie – Solange	Lecturer	*In availability*
24	EWANE Cécile Anne	Lecturer	In service
25	KOTUE TAPTUE Charles	Lecturer	In service
37	LUNGA Paul KEILAH	Lecturer	In service
26	MBONG ANGIE M. Mary Anne	Lecturer	In service
27	MOFOR née TEUGWA Clotilde	Lecturer	CE SEPMINESUP
28	NJAYOU Frederic Nico	Lecturer	In service
29	Palmer MASUMBE NETONGO	Lecturer	In service
30	SHANG Judith DZELAMBONG	Lecturer	In service
31	TCHANA KOUATCHOUA Angèle	Lecturer	In service
32	BEBEE Fadimatou	Lecturer	In service

33	DONGMO LEKAGNE Joseph Blaise	Lecturer	In service
34	FONKOUA Martin	Lecturer	In service
35	BEBOY EDJENGUELE Sara Nathalie	Assistant	In service
36	DAKOLE DABOY Charles	Assistant	In service
38	MANANGA Marlyse Joséphine	Assistant	In service
39	MBOUCHE FANMOE Marcelline Joëlle	Assistant	In service
40	PACHANGOU NSANGOU Sylvain	Assistant	In service
41	TIENTCHEU DJOKAM Leopold	Assistant	In service
2- DÉPARTMENT OF ANIMAL BIOLOGY AND PHYSIOLOGY ANIMAL (BPA) (44)			
1	BILONG BILONG Charles-Félix	Professor	Head of Department
2	DIMO Théophile	Professor	In service
3	DJIETO Lordon Champlain	Professor	In service
4	ESSOMBA née NTSAMA MBALLA	Professor	*VD FMSB*
5	FOMENA Abraham	Professor	In service
6	KAMTCHOUING Pierre	Professor	In service
7	NJAMEN Dieudonné	Professor	In service
8	NJIOKOU Flobert	Professor	In service
9	NOLA Moïse	Professor	In service
10	TAN Paul Vernyuy	Professor	In service
11	TCHUEM TCHUENTE Louis Albert	Professor	*Progr. Coord. MINSANTE*
12	AJEAGAH Gidéon AGHAINDOUM	Associate Professor	Cheif of Service DPER
13	DZEUFIET DJOMENI Paul Désiré	Associate Professor	In service
14	FOTO MENBOHAN Samuel	Associate Professor	In service
15	KAMGANG René	Associate Professor	*C.E. MINRESI*
16	KEKEUNOU Sévilor	Associate Professor	In service
17	MEGNEKOU Rosette	Associate Professor	In service
18	MONY NTONE Ruth	Associate Professor	In service
19	TOMBI Jeannette	Associate Professor	In service

20	ZEBAZE TOGOUET Serge Hubert	Associate Professor	In service
21	ALENE Désirée Chantal	Lecturer	In service
22	ATSAMO Albert Donatien	Lecturer	In service
23	BELLET EDIMO Oscar Roger	Lecturer	In service
24	BILANDA Danielle Claude	Lecturer	In service
25	DJIOGUE Séfirin	Lecturer	In service
26	DONFACK METCHI Mireille	Lecturer	In service
27	GOUNOUE KAMKUMO Raceline	Lecturer	In service
28	LEKEUFACK FOLEFACK Guy B.	Lecturer	In service
29	MAHOB Raymond Joseph	Lecturer	In service
30	MBENOUN MASSE Paul Serge	Lecturer	In service
31	MOUGANG Luciane Marlyse	Lecturer	In service
32	MVEYO NDANKEU Yves Patrick	Lecturer	In service
33	NGOUATEU KENFACK Omer Bébé	Lecturer	In service
34	NGUEGUIM TSOFACK Florence	Lecturer	In service
35	NGUEMBOK	Lecturer	In service
36	NJATSA Hermine épse MEGAPTCHE	Lecturer	In service
37	NJUA Clarisse Yafi	Lecturer	CD/UBa
38	NOAH EWOTI Olive Vivien	Lecturer	In service
39	TADU Zéphyrin	Lecturer	In service
40	YEDE	Lecturer	In service
41	ETEME ENAMA Serge	Assistant	In service
42	KANDEDA KAVAYE Antoine	Assistant	In service
43	KOGA MANG DOBARA	Assistant	In service
3-DEPARTMENT OF PLANT BIOLOGY AND PHYSIOLOGY (BPV) (26)			
1			
2	AMBANG Zachée	Professor	Vice Doyen/UYII
3	BELL Joseph Martin	Professor	In service
4	YOUMBI Emmanuel	Professor	Head of Department

5	MOSSEBO Dominique Claude	Professor	In service
5	BIYE Elvire Hortense	Associate Professor	In service
7	DJOCGOUE Pierre François	Associate Professor	In service
8	KENGNE NOUMSI Ives Magloire	Associate Professor	In service
9	MALLA Armand William	Associate Professor	In service
10	NDONGO BEKOLO	Associate Professor	*CE / MINRESI*
11	NGONKEU MAGAPTCHE Eddy L.	Associate Professor	In service
12	ZAPFACK Louis	Associate Professor	In service
13	ANGONI Hyacinthe	Lecturer	In service
14	MAHBOU SOMO TOUKAM Gabriel	Lecturer	In service
15	MBARGA BINDZI Marie Alain.	Lecturer	CT/Univ Dschang
16	MBOLO Marie	Lecturer	In service
17	NGODO MELINGUI Jean Baptiste	Lecturer	In service
34	NGALLE Hermine BILLE	Lecturer	In service
18	NGOUO Lucas Vincent	Lecturer	In service
19	NSOM ZAMO Annie Claude épse PIAL	Lecturer	*National Expert. / UNESCO*
39	TONFACK Libert Brice	Lecturer	In service
31	TSOATA Esaïe	Lecturer	In service
32	DJEUANI Astride Carole	Assistant	In service
33	MAFFO MAFFO Nicole Liliane	Assistant	In service
35	NGOMANDJE Christelle	Assistant	In service
36	NNANGA MEBENGA Ruth Laure	Assistant	In service
37	NOUKEU KOUAKAM Armelle	Assistant	In service
4-DEPARTMENT OF INORGANIC CHEMISTRY (CI) (33)			
1	AGWARA ONDOH Moïse	Professor	*Vice Rector Univ. Bamenda*
2	Florence UFI CHINJE épouse MELO	Professor	*VICE CHANCELLOR Univ. Ngaoundere*
3	GHOGOMU Paul MINGO	Professor	*Director of Cabinet PM*
4	LAMINSI Samuel	Professor	In service
5	NANSEU Charles Péguy	Professor	In service

5	NDIFON Peter TEKE	Professor	*ISI MINRESI/Head of Department*
7	NENWA Justin	Professor	In service
8	NGAMENI Emmanuel	Professor	*DEAN FS Univ. Dschang*
9	NJOPWOUO Daniel	Professor	In service
10	AVOM Jérôme	Associate Professor	*Director at IAI Gabon*
11	BABALE née DJAM DOUDOU	Associate Professor	*In Charge of Special duties P.R.*
12	DJOUFAC WOUMFO Emmanuel	Associate Professor	In service
13	ELIMBI Antoine	Associate Professor	In service
14	KEU KEUMEGNE MBOUGUEM Jean C.	Associate Professor	In service
15	KONG SAKEO	Associate Professor	*In Charge of Special duties at P.R.*
16	NDIKONTAR Maurice KOR	Associate Professor	*Vice-DEAN Univ. Bamenda*
17	NGOMO Horace MANGA	Associate Professor	*S.G. MINESUP*
18	NJIOMOU C. épse DJANGANG	Associate Professor	In service
19	YOUNANG Elie	Associate Professor	In service
20	ACAYANKA Elie	Lecturer	In service
21	BAIZOUMI ZOUA	Lecturer	*Chief of Unit MINTOUR*
22	EMADACK Alphonse	Lecturer	In service
23	GWET Simon – Pierre	Lecturer	In service
24	KAMGANG YOUBI Georges	Lecturer	In service
25	NDI Julius NSAMI	Lecturer	In service
26	NJOYA Dayirou	Lecturer	In service
27	PABOUDAM GBAMBIE A.	Lecturer	In service
28	SIGNING Pierre	Lecturer	In service
29	TCHAKOUTE KOUAMO Hervé	Lecturer	In service
30	BELIBI BELIBI Placide Désiré	Lecturer	In service
31	CHEUMANI YONA Arnaud M.	Lecturer	In service
32	NYAMEN Linda Dyorisse	Lecturer	In service
5-DEPARTMENT OF ORGANIC CHEMISTRY (CO) (35)			
1	DONGO Etienne	Professor	Vice-Dean/ DSSE
2	GHOGOMU TIH ROBERT RALPH	Professor	In service

3	MBAFOR Joseph	Professor	In service
4	NGADJUI TCHALEU B.	Professor	*Head of Dept FMBS*
5	NGOUELA Silvère Augustin	Professor	In service
6	NKENGFACK Augustin Ephraïm	Professor	Head of Department
7	NYASSE Barthélemy	Professor	*Head of Unit MINESUP*
8	PEGNYEMB Dieudonné Emmanuel	Professor	*Head of Unit MINESUP*
9	WANDJI Jean	Professor	In service
10	Alex de Théodore ATCHADE	Associate Professor	*CS Rectorate/UYI*
11	FOLEFOC Gabriel NGOSONG	Associate Professor	*Vice-Dean Univ. Buea*
12	KEUMEDJIO Félix	Associate Professor	In service
13	KOUAM Jacques	Associate Professor	In service
14	MBAZOA née DJAMA Céline	Associate Professor	In service
15	NOUNGOUE TCHAMO Diderot	Associate Professor	In service
16	TCHOUANKEU Jean-Claude	Associate Professor	*Vice-Rector/ UYII*
17	YANKEP Emmanuel	Associate Professor	In service
18	TCHUENDEM KENMOGNE Marguerite	Associate Professor	In service
19	TIH née NGO BILONG E. Anastasie	Associate Professor	In service
20	MKOUNGA Pierre	Associate Professor	In service
21	NGO MBING Joséphine	Associate Professor	In service
22	TABOPDA KUATE Turibio	Associate Professor	In service
23	AMBASSA Pantaleon	Lecturer	In service
24	EYONG Kenneth OBEN	Lecturer	In service
25	FOTSO WABO Ghislain	Lecturer	In service
26	KAMTO Eutrophe Le Doux	Lecturer	In service
27	KEUMOGNE Marguerite	Lecturer	In service
28	NGONO BIKOBO Dominique Serge	Lecturer	In service
29	NOTE LOUGBOT Olivier Placide	Lecturer	In service
30	OUAHOUO WACHE Blandine M.	Lecturer	In service
31	TABOPDA KUATE Turibio	Lecturer	In service
32	TAGATSING FOTSING Maurice	Lecturer	In service

33	ZONDENDEGOUMBA Ernestine	Lecturer	In service
34	NGOMO Orléans	Lecturer	In service
35	NGNINTEDO Dominique	Assistant	In service
6- COMPUTER SCIENCE DEPARTMENT (IN) (25)			
1.	FOTSO Pauline Laure	Professor	*Vice-Rector UDs*
2	ATSA ETOUNDI Roger	Professor	Head of Department
3	FOUDA NDJODO Marcel Laurent	Professor	*IA4-MINESUP/Head Dpt ENS*
4	TCHUENTE Maurice	Professor	*PCA UB*
5	NDOUNDAM René	Associate Professor	In service
6	KOUOKAM KOUOKAM E. A.n	Lecturer	In service
7	CHEDOM FOTSO Donatien	Lecturer	In service
8	MELATAGIA YONTA Paulin	Lecturer	In service
9	MOTO MPONG Serge Alain	Lecturer	In service
10	TINDO Gilbert	Lecturer	In service
11	TSOPZE Norbert	Lecturer	In service
12	WAKU KOUAMOU Jules	Lecturer	In service
13	ABESSOLO ALO'O Gislain	Assistant	In service
14	BAYEM Jacques Narcisse	Assistant	In service
15	DJOUWE MEFFEJA Merline Flore	Assistant	In service
16	DOMGA KOMGUEM Rodrigue	Assistant	In service
17	EBELE Serge	Assistant	In service
18	HAMZA Adamou	Assistant	In service
19	KAMDEM KENGNE Christiane	Assistant	In service
20	KAMGUEU Patrick Olivier	Assistant	In service
21	KENFACKDONGMO Clauvice V.	Assistant	In service
22	MEYEMDOU Nadège Sylvianne	Assistant	In service
23	MONTHE DJIADEU Valery M.	Assistant	In service
24	JIOMEKONG AZANZI Fidel	Assistant	In service
25	TAPAMO KENFACK Hyppolite	Assistant	In service
7-DEPARTMENT OF MATHEMATICS (MA) (35)			
1	BEKOLLE David	Professor	*Vice-Rector UN*

2	BITJONG NDOMBOL	Professor	*DAAC UYI*
3	DOSSA COSSY Marcel	Professor	In service
4	NGUETSENG Gabriel	Professor	*Head of CUTI UYI*
5	AYISSI Raoult Domingo	Associate Professor	Head of Department
5	EMVUDU WONO Yves S.	Associate Professor	*Head of Unit MINESUP*
7	NKUIMI JUGNIA Célestin	Associate Professor	In service
8	NOUNDJEU Pierre	Associate Professor	In service
9	TCHAPNDA NJABO Sophonie B.	Associate Professor	In service
10	TONGA Marcel	Associate Professor	In service
11	AGHOKENG JIOFACK Jean Gérard	Lecturer	In service
12	CHENDJOU Gilbert	Lecturer	In service
11	DOUANLA YONTA Hermann	Lecturer	In service
12	FOMEKONG Christophe	Lecturer	In service
13	KIANPI Maurice	Lecturer	In service
14	KIKI Maxime Armand	Lecturer	In service
15	MBAKOP Guy Merlin	Lecturer	In service
16	MBANG Joseph	Lecturer	In service
17	MBEHOU Mohamed	Lecturer	In service
18	MBELE BEDIMA Martin	Lecturer	In service
19	MENGUE MENGUE David Joe	Lecturer	In service
20	NGUEFACK Bertrand	Lecturer	In service
21	POLA DOUNDOU Emmanuel	Lecturer	In service
22	TAKAM SOH Patrice	Lecturer	In service
23	TCHANGANG Roger Duclos	Lecturer	In service
24	TCHOUNDJA Edgar Landry	Lecturer	In service
25	TIAYA TSAGUE N. Anne- Marie	Lecturer	In service
26	DJIADEU NGAHA Michel	Assistant	In service
27	MBIAKOP Hilaire George	Assistant	In service
28	NIMPA PEFOUKEU Romain	Assistant	In service
29	TANG AHANDA Barnabé	Assistant	*Cheif Serv. MINPLAMAT*

30	TETSADJIO TCHILEPECK Mesmin Erick	Assistant	In service
8-DEPARTMENT OF MICROBIOLOGY (MB) (13)			
1	ETOA François-Xavier	Professor	Vice Chancellor UDO/Head of Department
2	BOYOMO ONANA	Associate Professor	In service
3	ESSIA NGANG Jean Justin	Associate Professor	*Head of Division research IMPM*
4	NYEGUE Maximilienne Ascension	Associate Professor	In service
5	NWAGA Dieudonné M.	Associate Professor	In service
6	RIWOM Sara Honorine	Associate Professor	In service
7	SADO KAMDEM Sylvain Leroy	Associate Professor	In service
8	BODA Maurice	Lecturer	In service
9	ENO Anna Arey	Lecturer	In service
10	ESSONO OBOUGOU Germain Gabriel	Lecturer	In service
11	BOUGNOM Blaise Pascal	Lecturer	In service
12	NJIKI BIKOÏ Jacky	Assistant	In service
13	TCHIKOUA Roger	Assistant	In service
9-DEPARTMENT OF PHYSICS (PH) (41)			
1	ESSIMBI ZOBO Bernard	Professor	In service
2	KOFANE Timoléon Crépin	Professor	In service
3	NJOMO Donatien	Professor	In service
4	TABOD Charles TABOD	Professor	*DEAN/Ubda*
5	WOAFO Paul	Professor	In service
6	NDJAKA Jean Marie Bienvenu	Professor	Head of Department
7	PEMHA Elkana	Professor	In service
8	TCHAWOUA Clément	Professor	In service
9	BIYA MOTTO Frédéric	Associate Professor	*Dir.Gen. B. MEKIM*
10	BEN- BOLIE Germain Hubert	Associate Professor	In service
11	DJUIDJE KENMOE Gemaine épse ALOYEM KAZE	Associate Professor	In service

12	EKOBENA FOUDA Henri Paul	Associate Professor	*Head Dept UN*
14	NANA NBENDJO Blaise	Associate Professor	In service
15	NOUAYOU Robert	Associate Professor	In service
16	SIEWE SIEWE Martin	Associate Professor	In service
17	ZEKENG Serge Sylvain	Associate Professor	In service
18	EYEBE FOUDA Jean Sire	Associate Professor	In service
19	FEWO Serge Ibraïd	Associate Professor	In service
20	HONA Jacques	Associate Professor	In service
21	OUMAROU BOUBA	Associate Professor	*Recteur UYII*
22	BODO Bernard	Lecturer	In service
23	EDONGUE HERVAIS	Lecturer	In service
24	FOUEDJIO David	Lecturer	In service
25	MBANE BIOUELE	Lecturer	In service
26	MBINACK Clément	Lecturer	In service
27	MBONO SAMBA Yves Christian U.	Lecturer	In service
28	MBOUSSI NKOMIDDIO Aïssatou	Lecturer	In service
29	NDOP Joseph	Lecturer	In service
30	OBOUNOU Marcel	Lecturer	In service
31	SEIDOU	Lecturer	In service
32	SIMO Elie	Lecturer	In service
33	TABI Conrad Bertrand	Lecturer	In service
34	TCHOFFO Fidèle	Lecturer	In service
35	VONDOU DERBETINI Appolinaire	Lecturer	In service
36	WOULACHE Rosalie Laure	Lecturer	In service
37	ABDOURAHIMI	Lecturer	In service
38	CHAMANI Roméo	Lecturer	In service
39	ENYEGUE A NYAM Françoise épouse BELINGA	Lecturer	In service
40	WAKATA née BEYA Annie	Lecturer	*Cheif Serv. MINESUP*
10- DEPARTMENT OF SOIL SCIENCE (ST) (42)			
1	DJIGUI Paul Désiré	Professor	Head of Department

2	BITOM Dieudonné	Professor	Dean FS/ UDs
3	NDJIGUI Paul-Désiré	Professor	In service
4	NZENTI Jean-Paul	Professor	In service
5	FOUATEU Rose épse YONGUE	Associate Professor	In service
5	KAMGANG Pierre	Associate Professor	In service
7	MEDJO EKO Robert	Associate Professor	*Head Div. Rectorate UYI*
8	MVONDO ONDOA Joseph	Associate Professor	*S / Director MINVILLE*
9	NDAM NGOUPAYOU Jules-Remy	Associate Professor	In service
10	NGOS III Simon	Associate Professor	In service
11	NJILAH Isaac KONFOR	Associate Professor	In service
12	NKOUMBOU Charles	Associate Professor	In service
13	TEMDJIM Robert	Associate Professor	In service
14	YENE ATANGANA Joseph Q.	Associate Professor	*Head Div. MINEF*
15	ABOSSOLO née ANGUE Monique	Associate Professor	*Head Div. DAASR/ FS*
16	GHOGOMU Richard TANWI	Associate Professor	In service
17	MOUNDI Amidou	Associate Professor	*Head Div. MINMIDT*
18	ONANA Vincent	Associate Professor	In service
19	TCHOUANKOUE Jean-Pierre	Associate Professor	In service
20	ZO'O ZAME Philémon	Associate Professor	*S.G. MINTP*
21	ESSONO Jean	Lecturer	*C.E.A. MINES*
	FUH Calistus Gentry	Lecturer	*Sec. of State /MINMIDT*
22	GANNO Sylvestre	Lecturer	*In service*
23	LAMILEN BILLA Daniel	Lecturer	In service
24	MBIDA YEM	Lecturer	*CS/LABOGENIE*
25	MINYEM Dieudonné-Lucien	Lecturer	*Dir. of "Hydro-MIN" Project*
26	MOUAFO Lucas	Lecturer	In service
27	MOUNDI Amidou	Lecturer	*Head Div. MINIMDT*
28	NJOM Bernard de Lattre	Lecturer	In service
29	NGO BELNOUN Rose Noël	Lecturer	In service
30	NGO BIDJECK Louise Marie	Lecturer	In service
31	NGUEUTCHOUA Gabriel	Lecturer	In service

32	NYECK Bruno	Lecturer	In service
33	TCHAKOUNTE J. épse NOUMBEM	Lecturer	*CT/ MINRESI*
34	ANABA ONANA Achille Basile	Assistant	*In service*
36	METANG Victor	Assistant	*In service*
37	NOMO NEGUE Emmanuel	Assistant	In service
38	TCHAPTCHET TCHATO De P.	Assistant	In service
39	TEHNA Nathanaël	Assistant	In service
40	TEMGA Jean Pierre	Assistant	*In service*

Numerical breakdown of permanent teachers of the Faculty of Science of the University of Yaounde I

NUMBER OF TEACHERS					
DEPARTMENT	Professors	Associate Professors	Lecturers	Assistants	Total
B.C.	5 (1)	11 (3)	19 (12)	6 (4)	41 (20)
B.P.A	11 (1)	9 (3)	20 (9)	3 (0)	43 (13)
B.P.V	4 (0)	7 (0)	9 (4)	6 (6)	26 (10)
C.I.	9 (1)	10 (1)	13 (2)	0 (2)	32 (6)
C.O.	9 (0)	13 (3)	12 (4)	1 (0)	35 (7)
I.N.	4 (1)	1 (0)	7 (0)	13 (3)	25 (4)
M.A.	4 (0)	6 (0)	19 (1)	5 (0)	35 (1)
M.B.	2 (0)	3 (1)	8 (2)	0 (0)	13 (3)
P.H.	8 (0)	13 (2)	19 (3)	0 (0)	40 (5)
S.T.	4 (0)	16 (1)	14 (4)	7 (0)	40 (5)
Total	60 (4)	89 (14)	140 (41)	41 (15)	*340 (73)*

Total : 340 (73) dont
- Professors 60 (4)
- Associate Professors 89 (14)
- Lecturers 140 (41)
- Assistants 41 (15)
- ( ) = Number of women.

The DEAN of the Faculty of Science

DEDICATION

This thesis is dedicated to my lovely family:
my wife ABANG Marcelline NKENGLACK and my two children
FOMUMBOD Daelan ABANG and FOMUMBOD Dasha SEH
for their endeorance and patience.

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LIST OF ABBREVIATIONS

ANKE: Aqueous Neem Kernel Extract

AUIPC: Area Under the Infestation Pressure Curve

AVRDC: Asian Vegetable Research and Development Center (The World Vegetable Center)

bp: base pair

Cox I: Cytochrome C Oxidase Sub-unit I

DNA: Deoxyribonucleic Acid

EF: Elongation Factor

IC: Integrated Control

IITA: International Institute of Tropical Agriculture

IPM: Integrated Pest Management

NBPGR: National Bureau of Plant Genetic Resources

NCBI: National Center for Biotechnology Information

NSKE: Neem Seed Kernel Extract

O.D: Optical density

OPs: Organophosphates

PCR: Polymerase Chain Reaction

RAE: Retinol Activity Equivalents

TE: Tris EDTA (Ethylenediamine Tetra-acetic Acid)

MINADER: Ministry of Agriculture and Rural Development

Vat: Virus aphid transmission

WAS: Weeks After Sowing

WCA: West and Central Africa

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SUMMARY

Aphis gossypii Glover (Hemiptera: Aphididae) is one of the major pests of okra (*Abelmoschus* spp). On one hand, direct damages due to its feeding habit results in curling and deformation of young leaves. On the other hand, indirect damages are caused because of honeydew secreted on fruits and leaves with, which in turn may promot growth of black sooty mould. The black sooty mouls stain and reduce fruit and leaf quality and reduce photosynthetic activity. In addition, honeydew attracts ants that fend off natural enemies of Hemipterans. The severity of aphid infestation has led to widespread use of chemical pesticides for its control with adverse effect that it also eliminates the natural enemies. Pests including aphids such as *A. gossypii* are becoming resistant to pesticides. Most vegetable farmers in Cameroon accept that they use chemical pesticides, and are equally willing to accept new varieties that are resistant to pests and diseases, to minimize the use of pesticides. The objective of this work was to identify aphid-resistant okra germplasm for a better management of *A. gossypii*.

Screening trials were conducted under natural field conditions without pesticide application. Aphid infestations per variety were directly scored on one leaf per stratum on three strata of five plants randomly selected. The number of aphids was recorded using the following scale: 0 = no aphids present; 1 = 1 to 10 aphids per leaf; 2 = 11 to 100 aphids per leaf; 3 = 101 to 500 aphids per leaf; and 4 = >500 aphids per leaf. Phenotypic structures and secondary metabolites that could affect the life traits of Aphis gossypii were analysed. In the case of phenotypic structures, trichome density, hardness and chlorophyll content of okra leaves were taken into consideration. Concerning secondary metabolites, leaf contents in total phenols, total tannins, free amino acides, total sugars, reducing sugars, total nitrogen and potassium were considered. The implications of mechanisms of tolerance, antibiosis and antixenosis were evaluated in the analysis of resistance of plants of *Abelmoschus* spp.

Nine okra accessions were therefore identified as resistant or moderately resistant to *A. gossypii*. The most resistant ones were VI041210, VI057245 and Gombo caféier. The farmers' check Kirikou and VI060694 were the most tolerant. Resistant accessions produced fewer pods than susceptible and tolerant accessions. In this study, non-preference (antixenosis) was not a category of resistance. The non-discrimination between susceptible and resistant accessions in aphid settling behaviour indicates that phenotypic structures and plant metabolites did not influence attraction and settling behaviour. The trichome density was highest on the leaves of the top stratum, higher at the middle stratum and lower at the bottom; it was lower on VI060794 and the farmers' check, Kirikou, at all plant strata, and may favour infestation of these susceptible accessions. The current study

revealed the role of total nitrogen content in leaves leading to the susceptibility of okra accessions to aphids. VI060794 that was the most susceptible in Taiwan in 2013 and in the second season of the confirmatory screening trial in Cameroon in 2014 had significantly higher leaf Nitrogen content than in other accessions. Constitutively, the role of free amino acids, tannins and total phenols in imparting resistance against *A. gossypii* in the identified okra accessions during our study is inconclusive. Biochemical studies of accessions of okra at 6 and 10 weeks after sowing showed that total phenols and tannins content changed following aphid infestation. Total tannins increased in the resistant accessions and reduced in Kirikou, the susceptible farmers' check at all plant growth stages. The total sugars, potassium and reducing sugars played a role in offering resistance in plants with or without aphids. As a susceptible accession, VI060794 had higher nitrogen content significantly at vegetative stage following aphid infestation and at reproductive growth of plant even when plants were not infested. The farmers' check Kirikou that was one of the most susceptible to aphids had the highest intrinsic rate of natural increase of aphid population, which was significantly different from that of VI057245, one of the most resistant accessions during confirmatory and multilocation trials. When plants were previously infested with aphids at vegetative and reproductive stages, the developmental time was significantly longer on VI041210 than on all accessions except VI060688 at vegetative growth. No mortality of aphids was observed on VI033805, VI033824 and on the farmer's check Kirikou. Results from the multilocation trials indicated that the farmers' varieties were more susceptible to aphids than most of the selected resistant accessions, across all agro-ecological zones.

VI057245 and VI036213 are suitable for resistance to aphids in the western highland; VI060818, VI060794 and VI039614 in the monomodal humid rain forest; VI060794, VI057245, VI051114 and Gombo caféier for the bimodal humid rain forest, VI060818 and VI041210 in the Sudano-Sahelian region. VI060794 was also the most yielding in all ecozones in Cameroon and with some acceptable level of resistance. We recommend the following accessions for the presence of resistant traits: VI041210, VI051114, VI033824, VI057245 and VI036213 for leaf trichomes; VI051114 and VI036213 for fruit size; VI041210, VI060794 and Gombo cafiere for plant vigour. VI041210, VI057245 and Gombo cafiére for higher secondary metabolites and lower plant nutrients contents leading to antibiosis. VI060794 presents superior qualities in terms of yields and management of aphids.. It will also be interesting to study the genotypes of the selected accessions to identify genes associated with resistance to *A. gossypii*.

Key words: *Aphis gossypii*, okra, resistant accessions, aphids

RESUME

Aphis gossypii Glover (Hemiptera : Aphididae) est l'un des principaux ravageurs du gombo (*Abelmoschus* spp.). Les dégâts directs qu'il cause (dus à son mode d'alimentation) se matérialisent par le rabougrissement et la déformation des jeunes feuilles des plantes hôtes. Par contre, les dégâts indirects se traduisent par le recouvrement des fruits et des feuilles par du miellat qui, à son tour, peut provoquer le développement des fumagines. De plus, ce miellat attire les fourmis qui repoussent les ennemis naturels des hemiptères. En champs, de fortes infestations par des pucerons conduisent à une utilisation généralisée des pesticides chimiques qui, malheureusement, éliminent les ennemis naturels des homoptères. Par conséquent, les ravageurs tels que les pucerons, y compris *A. gossypii*, sont devenus résistants aux pesticides. Au Cameroun, pour minimiser l'utilisation des pesticides, la plupart des maraîchers ont toujours souhaité adopter de nouvelles variétés résistantes de cultures. Ainsi, l'objectif de ce travail était d'identifier le germoplasme du gombo résistant aux pucerons pour une meilleure gestion d'*A. gossypii*.

Les essais de screening ont été menés en champs dans les conditions naturelles, en absence de pesticide. Les infestations des plants ont été étudiées directement sur une feuille pour chacune des trois strates de cinq plantes choisies au hazard, et par variété. Le nombre des aphides a été evalueé en utilisant l'échelle suivante : 0 = absence, 1 = 1 à 10 aphides, 2 = 11 à 100 aphides, 3 = 101 à 500 aphides, et 4 = >500 aphides. Les structures phénotypiques et les métabolites secondaires qui peuvent influencer les traits de vie d'*A. gossypii* ont été analysés. Dans le cas des structures phénotypiques, la teneur en chlorophylle, la pilosité et la texture des feuilles du gombo ont été prises en compte. Pour ce qui est des métabolites, les teneurs des feuilles en phénols totaux, tanins totaux, acides aminés libres, sucres totaux, sucres réducteurs, azote et potassium ont été considérées. L'implication de mécanismes de résistance telles que la tolérance, l'antibiose et l'anti-xénose a été évaluée dans l'analyse de résistance *d'Abelmoschus* spp.

Neuf variétés de gombo ont été identifiées comme résistantes ou modérément résistantes à *A. gossypii* ; celles des accessions codées VI041210, VI057245 et le Gombo caféier se sont révélées plus résistantes à cette espèce d'insecte ; par contre, la variété "Kirikou", utilisée comme témoin dans ce travail, et VI060794 en ont été plus tolérantes. Les accessions résistantes ont produit moins de fruits comparées à celles qui étaient sensibles ou tolérantes. Ce travail a montré l'absence d'une préférence d'une variété du gombo par *A. gossypii*, soulignant donc que l'antixénose n'est pas une catégorie de résistance des variétés de cette culture. Les accessions sensibles et résistantes n'ont révélé aucune différence du point de vue attraction et comportement de le chois de la plante-hote chez les pucerons ; il en découle que les structures phénotypiques et les métabolites secondaires de gombo (*Abelmoschus* spp.) n'ont pas completement influencé les traits de vie d'*A. gossypii*. La pilosité des feuilles a été plus élevée sur les feuilles de la strate supérieure de la plante de la plante, moyenne puis faible respectivement au niveau des strates médiane et basale de la plante. La faible pilosité des feuilles pour les variétés VI060794

et Kirikou a favorisé leur infestation à toutes les strates de la plante. Le rôle de la teneur des feuilles en azote concernant la sensibilité des plantes a été mis en évidence. Ainsi VI060794, l'accession la plus sensible à Taïwan en 2013 et au Cameroun en 2014, a eu une teneur en azote significativement plus élevée dans ses feuilles. Cette variété sensible a présenté une teneur en azote significativement plus élevée aux stades végétatif et reproductif de la plante et quel que soit son statut infesté ou indemne. La population de pucerons s'est alors accrue sur la variété "Kirikou". Les acides aminés libres, les tanins et les phénols totaux n'ont pas semblé jouer un rôle décisif dans la résistance du gombo contre *A. gossypii*. Les études biochimiques des accessions d'*Abelmoschus* spp. à 6 et à 10 semaines après semis, ont montré que la teneur en phénols et celle en tanins sont modifiables suivant les niveaux d'infestation par les pucerons. A tous les stades de croissance des plants, la teneur en tanins totaux a augmenté dans les accessions résistantes alors qu'elle était réduite dans la variété "Kirikou". Le potassium, les sucres totaux et les sucres réducteurs ont joué un rôle dans la résistance des plantes hébergeant ou non les pucerons. Lorsque les plantes ont été infestées de pucerons aux stades végétatif et reproductif, la durée de développement des nymphes a été significativement plus longue sur VI041210 comparée à toutes les accessions, mais seulement au stade reproductif pour ces dernières. Aucune mortalité des pucerons n'a été observée sur les variétés Kirikou, VI033805 et VI033824. Les résultats des essais multi-sites ont indiqué que les variétés utilisées par les agriculteurs sont plus sensibles que la plupart de celles qui ont été étudiées.

Les variétés VI057245 et VI036213 ont résisté aux pucerons dans les hautes terres de l'Ouest Cameroun, de même que VI060818, VI060794 et VI039614 dans la forêt tropicale humide monomodale, VI060794, VI057245, VI051114 et Gombo caféier dans la forêt tropicale humide et bimodale, VI060818 et VI041210 dans la région soudano-sahélienne. L'accession VI060794 est apparue plus rentable dans les différents sites, avec une résistance appréciable. En raison de leurs caractères phénotypiques et biochimiques, les variétés VI041210, VI051114, VI033824, VI057245 et VI036213 sont recommandées du fait de leurs pilosités des feuilles élevées, VI051114 et VI036213 pour une production des fruits de grande taille, VI041210, VI060794 et Gombo caféier pour la vigueur de leurs plants, VI041210, VI057245 et Gombo caféier pour la teneur élevée en métabolites secondaires utiles dans l'antibiose. Enfin, VI060794 présente des qualités supérieures en termes de rendement et de bonne gestion contre des pucerons. Il sera également intéressant d'étudier les génotypes des accessions sélectionnées pour identifier les gènes associés à la résistance à A. gossypii.

Mots clès : *Aphis gossypii*, gombo, accessions résistantes, pucerons.

INTRODUCTION

Insect-plant interaction and host plant resistance

The earth's vegetation cover is mostly green because what we see is mostly plants. However, there are more herbivorous insect species than the diversity of plant species on our planet. According to Hairston *et al.* (1960), this observation implies that herbivores are still too few to consume all the vegetation available. Murdoch (1966) suggested that perhaps plants have sufficiently defended themselves against pests. There are about 230-422000 flowering plant species interacting with 2 to 30 million insect species (Kessler, 2006). More than 400000 of the latter are described as phytophagous (Mitter *et al.,* 1991). The evolution of aphid has been described as strongly shaped by dependence on their host plants. About 99% of aphids being specialists, associated with one or just a few closely related plant species and 10% of the species regularly switch between two host plant species (primary and secondary host) during the seasons (Vilcinskas, 2016). Plants survive by developing new defense processes and reproduce while insects are constantly evolving new processes to overcome plant defences. This is the base of the co-evolution theory proposed by Ehrlich and Raven (1964). Despite the acceptance of this theory by researchers, it is important to indicate that certain plant defense compounds experience opposing selection pressure by different enemies and that major defensive barriers evolve in response to a diverse assemblage of herbivores and other biotic and abiotic factors (Stowe, 1998). This 'war' has chemical and physical components (manifested by texture, morphology, taste, odour, colour and size of insects and plants) may involve exploiting the abilities of outside predators that have evolved to take advantage of these conflicts. In addition, both plants and insects are under environmental pressures that have an impact on thier interactions (Panda and Khush, 1995).

The two broad categories of insect-plant interactions are herbivory (phytophagy) and mutualism. In the former, there exist three stages of interaction (pre-entry, entry, and colonization) (Walling, 2008). These stages will form the framework of resistance against herbivores conferred to host plants. Resistance is a relative property, based on the comparative reaction to the insect pest, by resistant and susceptible plants grown under similar conditions. The following types of resistance exist as described by Kogan and Paxton (1983). Plant resistance to insects is the genetically inherited qualities that result in a plant of one variety or species being less damaged than a susceptible plant lacking these qualities. Pseudo or false resistance in susceptible plants is resistance due to early planting, low levels of insect infestation, temperature

differences, day length, soil chemistry and plant or soil water content. Associational resistance refers to a normally susceptible plant growing in association with a resistant plant, and deriving protection from insect predation. Induced resistance, which is the enhancement of a plant's pest defense system in response to external physical or chemical stimuli (Kogan and Paxton 1983), occurs in many crops due to the elicitation of endogenous plant metabolites (Pearce *et al.*, 1991). Plants resistance to herbivores had long been categorized into three mechanisms: antixenosis, antibiosis, and tolerance (Painter, 1951). The term "mechanisms" of resistance was replaced by Kogan and Ortman (1978) with the term "categories" of resistance. Horber (1980) called the three as functional categories while Smith (1989) termed them functional modalities of resistance.

The resistance due to negative effects of a plant on the biology of an insect attempting to use it as a host is called antibiosis (Smith, 1989). Painter (1951) stated that antibiosis refers to the adverse effects on insect life history when a resistant plant variety is used as a food source. These effects can vary from small to lethal. Metcalf and Luckman (1994) gave several examples of antibiotic effects of hosts on insects: irregular growth rate and behaviour, malformation, decreased fecundity, reduced fertility, and death. Non-preference or Antixenosis (Kogan and Ortman, 1978) means simply that a given plant is not a preferred host of an insect for feeding and oviposition. Smith (1989) stated that antixenosis describes the inability of a plant to serve as a host to particular herbivore insect. The third category of resistance is tolerance; this is the ability of the plant to withstand insect damage and continue to grow and produce. The expression of tolerance is determined by the inherent genetic ability of a plant to outgrow an insect infestation or to recover and add new growth after the destruction or removal of damaged tissues (Smith, 1989). Factors affecting tolerance include plant vigour and regrowth of damaged tissues (Metcalf and Luckman, 1994). The mechinisms involved are six physiological (increased net photosynthetic rate after herbivory, high relative growth rates, increased branching or tillering, pre-existing high levels of carbon storage in roots, increased resource allocation from root to shoot after damage (Strauss and Agrawal, 1999) and up-regulation of detoxification mechanisms as a response to counteract harmful effects of herbivory (Koch *et al.*, 2016). Possible morphological mechanisms include protected meristems, number of meristems, and developmental plasticity (Rosenthal and Kotanen, 1994). However, it is expected that these mechanisms should translate into farmers' benefits.

The chemicals and morphological characteristics of plants are the bases of their resistance to insect (Khan, 1994). Both chemical and morphological plant defenses mediate resistance to insect pests through mechanisms of resistance such as olfactory repellents, feeding or oviposition deterrents, and toxins, or the absence of feeding or oviposition stimulants. Allelochemicals produce an unfavourable taste or smell for the insect. In addition, plants produce volatile organic compounds especially during pest infestation to repel herbivores, and to attract beneficial organisms such as predators, parasitoids and pollinators (Dicke and Van Loon, 2000; De Moraes, 2001; Kessler and Baldwin, 2001). Morphological defences are structural features of the plant, such as pubescence, that are unfavourable for insects (Zarpas *et al.*, 2006). Hosts with some unfavourable characteristics such as tall, open canopy, smooth leaves (Hector and Hodkinson, 1989; Nibouche *et al.,* 2008), red coloured varieties (Matthews and Tunstall, 1994) are always less severely attacked by arthropod pests, especially *Aphis gossypii* Glover (Hemiptera: Aphididae). Morphological or structural characteristics such as silica content, leaf toughness and size, deceptive plant structures, also play a vital role in enhancing plant resistance (Deguine and Hau, 2001). These characteristics influence aphids' settling and feeding behaviour. For instance, after 72 hours of infestation, most of the *A. gossypii* left the leaves of resistant melon plants, since they found them unsuitable for feeding and colonization (Soria *et al.*, 2000). On a virus aphid transmission (*Vat*)-resistant melon plant, *A. gossypii* seldom reached the phloem or stopped feeding in phloem when reached and then starved (Chen *et al*., 1996; Klingler *et al.*, 1998). Plants produce secondary and primary metabolites that are involved in antibiosis. Secondary metabolites can be divided into three chemically distinct groups *viz*: Terpenes, Phenolics, N and S containing compounds (Mazid *et al.*, 2011). Terpenoids, which are common in trees are also used as active ingredients of pesticides; phenols, most abundantly tannins act as toxins, repellents, and bind insect salivary proteins (Chandramani *et al.*, 2009); nitrogen and sulphur containing secondary metabolites such as alkaloids and non-protein amino acids are also protein inhibitors, deactivators, toxins and irritants. However, sulphur-containing compounds offer defence against pathogens. Primary metabolites include proteins (amino acids) and carbohydrates (sugars) and are important in the growth and development of animals and plants. The levels of free amino acids and sugars may partially determine the likelihood of infestation by *A. gossypii* (Deguine and Hau, 2001). An excess of nitrogen (N) or deficiency of potassium (K) can lead to higher accumulation of amino acids that, in turn, can cause higher attack rate by sucking insects (Jansson and Ekbom,

2002). Plant nitrogen is also an indicator of food quality and host selection by *Aphis gossypii* (Mattson, 1980; Slosser *et al.,* 1989).

Plant defence was generally assumed constitutive (always present in the plant), until recently when it was recognized that some of the defence traits and processes change, as an induced response to pest attack and damage (Khattab, 2007; Wilson *et al.,* 2011). Each type of defense can be either constitutive (always present in the plant), or induced (produced in reaction to damage or stress caused by external physical injury). Induced responses that reduce herbivore survival, reproduction or preference for a host, are termed induced resistance. Some induced responses may cause the plants to become more vulnerable to the target pest or to other potential dangers. They do not affect the plant. However, induced responses that decrease the plant fitness for subsequent herbivore attack are termed induced defence (Khattab, 2007). They defend either by repelling the pests or by attracting their natural enemies. The plant is affected but not the pest. Thus, induced responses are plastic traits that vary according to the environment and may or may not affect herbivores directly. They may or may not benefit the plants when attacked by herbivores. Plastic traits are not genetically fixed, but the ability to show plastic traits may be due to the genes control (Schlicting, 1986; Sultan, 1987; Bradshaw and Hardwick, 1989). Plants respond to herbivore attack through structural barriers, toxic chemicals, and attraction of natural enemies of the target pests (Hanley *et al.,* 2007; Howe and Jander, 2008; Karban, 2011).Defense mechanisms represent direct and indirect resistance and may be present constitutively or induced after damage by the herbivores.

Most aphid-resistant genes identified to date are restricted in their effectiveness to single aphid species, or even to particular biotypes. The cotton or melon aphid, *A. gossypii*, reproduces very rapidly and under these conditions, new biotypes can be formed very quickly. A biotype is an insect population capable of damaging and surviving on plants previously known to be resistant to populations of the same species (Metcalf and Luckman, 1994). For example, resistance in cotton appears to be restricted to *A. gossypii* only (Klingler *et al.,* 1998). *Medicago truncatula* cultivars that are resistant to *Acyrthosiphon kondoi* and the spotted alfalfa aphid *Therioaphis trifolii* did not affect the infestation by *Myzus persicae* and cowpea aphid (*A. craccivora*) (Gao *et al.,* 2007). Resistance to aphids appears to be species-specific; hence, it is important to confirm the species and/or biotypes of the target aphid population while selecting

resistant cultivars. Resistant crops are necessary to slow down pest problems on vegetables in general and okra in particular (Kumar *et al.,* 2010; Leke, 2010).

Problem statement of the study

Okra had been considered a minor crop until recent interest from medical experts, because of the presence of viscous fibres, which offer potentials for healthier diets (Duzyaman, 1997; Kendall and Jenkins, 2004). Okra is cultivated mainly for immature pods consumed fresh or dried, and added to soup, depending on the location. The pods contribute viscous fibres to the diet (Kendall and Jenkins, 2004) and the viscosity eases consumption of food (Schippers, 2000). In medicine, mucilage serves as a plasma replacement or blood volume expander, and for cholesterol reduction (Markose and Peter, 1990; Benchasri, 2012). Industrially, mucilage is used to glace papers, roasted seed added to coffee or as a coffee substitute in confectioneries (Markose and Peter, 1990). Increasing okra production can diversify vegetable production systems in sub-Saharan Africa and help improve diets (Hughes, 2009). The worldwide production of okra is estimated at 8.69 million tonnes annually at a yield of 7868 tonnes per ha. India is the highest producer in the world (73%), followed by Nigeria (13%). West Africa accounts for 76% of production in Africa; this continent produces 1.84 million tonnes annually with a yield of 3606 tonnes per hectare. In Cameroon, annual production stands at 72661 tonnes per year with a yield of 3027 tonnes per hectare below both Africa and world yields (FAOSTAT, 2015).

Okra cultivation faces many challenges including photoperiod sensitivity and cold temperatures that limit year-round availability of fresh pods, shelf life, fiber/mucilage content, and pest resistance, tomato fruit worm and begomoviruses (Kumar *et al.,* 2010). The cotton aphid, *Aphis gossypii* is one of the major pests of okra, particularly in tropical and subtropical regions (Kersting *et al.,* 1999), including Cameroon (Kekeunou *et al.,* 2006). *A. gossypii* occupies the top position among pests of vegetables including okra in Cameroon (Abang *et al.,* 2014). Aphids have a short life cycle but an extremely high reproductive rate. They reproduce throughout the year both parthenogenetically and sexually. Heavily infested okra plants commonly show distorted and stunted leaves and reduced fruit set (Wanja *et al*., 2001). *A. gossypii* damages either directly, by feeding which results in curling and deformation of young leaves and twigs, or indirectly by contaminating the fruits and leaves with honeydew that in turn may cause growth of black sooty mould that inhibits photosynthesis, and thus causing substantial

yield loss (Capinera, 2005). Yield losses can be up to 57% (Shannag *et al.,* 2007) when aphid infestation is exceedingly higher (>1000 aphids per plant) (Mohamed-Ahmed, 2000; Nderitu *et al.,* 2008). There can also be 100% yield loss if the attack is at the seedling stage (Doumbia and Seif, 2008). In addition, honeydew attracts ants that fend off natural enemies of Hemipterans (Yokomi and Tang, 1995).

The severity of aphid infestation has led to widespread use of chemical pesticides that also eliminate the natural enemies. Okra occupies the fourth position after tomato, hot pepper and African nightshade in consumption of chemical pesticides in Cameroon among the vegetable crops (Abang *et al.,* 2013). Pests including aphids are becoming resistant to pesticides and *A. gossypii* has developed resistance to carbamates, organophosphates, pyrethroids, and neonicotinoids (Denholm *et al.,* 2002; Wang *et al.,* 2002; Andrew *et al.,* 2006; Tabacian *et al*., 2011). Recent studies in Cameroon (Abang *et al.,* 2014) showed that 78% of vegetable farmers still use traditional varieties of vegetables that are susceptible to pests. While 90% accepted that they use chemical pesticides, an equal percentage indicated their willingness to accept new varieties that are resistant to pests and diseases to minimize the use of pesticides in okra production. Okra possesses chemical and physical properties that could resist pests. Some reports have confirmed the availability of aphid-resistant okra genotypes (Sumathi, 2005; Anitha and Nandihalli, 2009). However, most of these reports were based on a few local genotypes. For instance, only 15 local cultivars were screened in Tamil Nadu, India by Sumathi (2005). Cultivars such as Varsha Uphar and Arka Anamika were found to be moderately resistant. Anitha and Nandihalli (2009) evaluated only seven cultivated okra lines (mostly hybrids) for their resistance to aphid. No studies were carried out to elucidate the bases of resistance. Hence, there has been no concerted effort to identify aphid-resistant genotypes from a broader gene pool across the globe. The Asian Vegetable Research and Development Center (AVRDC) Genebank, the world's largest public sector vegetable germplasm collection, conserves more than 900 accessions of *Abelmoschus* spp., which offer broader gene pool required for a robust screening for resistance to aphid infestation. Hence, the current study was carried out to identify aphid-resistant okra accession(s) from this broad gene pool.

Hypothesis and objectives

Hypothesis

The use of host plant resistance in pest management will be studied based on the following hypotheses:

- most aphid-resistant genes identified to date are restricted in their effectiveness to single aphid species, or even to particular biotypes (Klingler *et al.*, 1998);
- phenetic traits of okra can affect aphid preference of okra varieties for feeding and oviposition. Trichomes have either adverse (Zarpas *et al.*, 2006) or positive effects (Nibouche *et al.*, 2008) on resistance to *Aphis gossypii*. Morphological or structural characteristics such as silica content, leaf toughness and size, and deceptive plant structures also play a vital role in enhancing plant resistance (Deguine and Hau, 2001). These morphological characters influence aphids' settling and feeding behaviour;
- okra produces secondary metabolites including terpenoid, phenols and tannins that defend the plant against aphids (Chandramani *et al.*, 2009). Primary metabolites and some nutrients favour aphid infestation on okra. The levels of certain components such as amino acids and sugars in a host may partially determine the likelihood of *Aphis gossypii* infestation (Deguine and Hau, 2001). Plant nitrogen level is an indicator of food quality and host selection by *A. gossypii* (Mattson, 1980; Slosser *et al.*, 1989);
- plant resistance, achieved using plant chemistry and phenetic traits, against herbivores has three mechanisms: antixenosis, antibiosis, and tolerance (Painter, 1951).

Objectives

The main objective is to select aphid-resistant okra germplasm for the management of *A. gossypii* in the tropics.

The specific objectives of this study are to:

- assess the genetic diversity of *A. gossypii* that occurs on okra in Cameroon and Taiwan;
- identify the okra accessions that are resistant to *A. gossypii*;
- study the effects of biophysical and biochemical (constitutive and feeding induced) properties of the okra varieties on resistance or susceptibility to aphids;

- assess the mechanisms of resistance (antixenotic, antibiotic and tolerance) of selected okra accessions;
- evaluate yield and ecological performance of identified resistant accessions.

CHAPTER I: LITERATURE REVIEW

I.1. Okra (*Abelmoschus* spp.)

I.1.1. Classification and taxonomy

Okra was first included in genus *Hibiscus*, section *Abelmoschus* in the family Malvaceae (Linnaeus, 1753). The section *Abelmoschus* was subsequently proposed to be raised to the rank of genus. However, most authors treated it as a section of *Hibiscus* spp. In 1924, Hochreutiner described 14 species and re-established the genus *Abelmoschus* of Medikus stating that calyx, corolla and stamens are fused together or connate at the base and caduceus or fall as one piece after anthesis, whereas in the case of *Hibiscus,* these are distinct (Kundu and Biswas, 1973; Aladele *et al.,* 2008). Six species were reported during the taxonomical revision undertaken by van Borssum Waalkes (1966) and Bates (1968), while Paul and Nair (1988) identified seven. With some minor changes to these reports nine species were adopted at the International Okra Workshop held at National Bureau of Plant Genetic Resources (NBPGR) in Delhi 1990 (IBPGR, 1991). Three varieties of *Abelmoschus angulosus* Wall. ex Wight and Arn., viz., var. angulosus, var. grandiflorus Thwaites and var. purpureus Thwaites are reported from India (Sivarajan *et al.*, 1994; Sivarajan and Pradeep, 1996). Recently, John *et al.* (2012) described a new species, *Ab. enbeepeegearense* from the Western Ghats and Sutar *et al.* (2013) described *Ab. palianus* from Chhattisgarh, India. Thus, presently there are eleven species described by Sutar *et al.* (2013) as below.

Kingdom: Plantae (Autotrophic)

Division: Tracheophyta (With vascular system)

Class: Magnoliopsida (Dicotyledonous)

Order: Malvales (leaves with palmate venation and sepals joined)

Family: Malvaceae (Stellate hairs on the young parts and mucilaginous juice present)

Genus: *Abelmoschus* (peculiar spathaceous calyx splitting to one-side)
Species: (epicalyx size and number, fruit size and morphology)

- *Abelmoschus moschatus* Medikus;
- *Abelmoschus manihot* (L.) Medikus;
- *Abelmoschus tetraphyllus* (Roxb. ex Hornem.) Borss var. tetraphyllus var. pungens ;
- *Abelmoschus esculentus* (L.) Moench;
- *Abelmoschus tuberculatus* Pal Singh;

- *Abelmoschus ficulneus* (L.) Wight Arn. ex. Wight;
- *Abelmoschus crinitus* Wall;
- *Abelmoschus angulosus* Wall. ex Wight and Arn. var. grandiflorus Thwaites var. angulosus var.purpureus Thwaites;
- *Abelmoschus caillei* (A. Chev.) Stevels;
- *Abelmoschus palianus* (S. P. Sutar, K.V.Bhat S.R.Yadav);
- *Abelmoschus enbeepeegearense* (J. John *et al*.).

I.1.2. Origin and Ecology of okra

I.1.2.1. Origin

The genus *Abelmoschus* consists of eleven species of which five occur in Africa: *Abelmoschus moschatus, Ab. manihot, Ab. caillei, Ab. esculentus* and *Ab. ficulneus* (Schippers, 2002). Within these species, numerous cultivars exist that vary in horticultural traits (Tindall, 1983). There are four known domesticated species of *Abelmoschus*; among these, *Ab. esculentus* are most widely cultivated in South and Southeast Asia, Africa, and the southern USA. In the humid zone of West Central Africa, *Abelmoschus caillei,* with a longer production cycle, is also cultivated (Siemonsma, 1982). Plants of *Ab. manihot* sometimes fail to flower and this species is extensively cultivated for leaves in Papua New Guinea (Hamon and Sloten, 1995), Solomon Islands and other South Pacific Islands (Keatinge, 2009). The fourth domesticated species, *Ab. Moschatus,* is cultivated for its seed, which is used for ambrette in India and several animism practices in South Togo and Benin (Hamon and Sloten, 1995). The genus *Abelmoschus* originated in South-East Asia (Siemonsma and Hamon, 2004). Nonetheless, *Ab. caillei, Ab. esculentus* as well as *Ab. ficulneus* are considered indigenous to Africa, while *Ab. moschatus* and *Ab. manihot* were introduced from Asia to Africa (Schippers, 2002). In fact, *Ab. caillei* is a cultigen, which occurs mainly in West and Central Africa. It has been reported from Guinea to Nigeria in West Africa, in Cameroon, Gabon and DR Congo in Central Africa, and in Uganda in East Africa. Its distribution is restricted to humid and per-humid climates in Africa, between 12°N and 12°S, most commonly between 5°N and 10°N, whereas the common okra *Ab. esculentus* (L.) Moench can be found worldwide throughout the tropics, subtropics and warm temperate regions (Siemonsma and Hamon, 2004). For *Ab. esculentus*, although an origin in the Sahara region is favoured by some reports and a northern Indian origin for others, there exists a

far greater diversity in this species in Africa than in Asia (Schippers, 2002). Similarly, Siemonsma and Piluek (1994) stated *Ab. esculentus* to be a cultigen of uncertain origin.

I.1.2.2. Ecology

The two most important okra species in Africa are *Abelmoschus esculentus* and *Abelmoschus caillei*. The latter is mainly found in the humid coastal zones of West and Central Africa, as well as in an area that extends from southern Senegal to southern Democratic Republic of Congo and up to Uganda (Schippers, 2002; Siemonsma and Hamon, 2004) (Figure 1a).

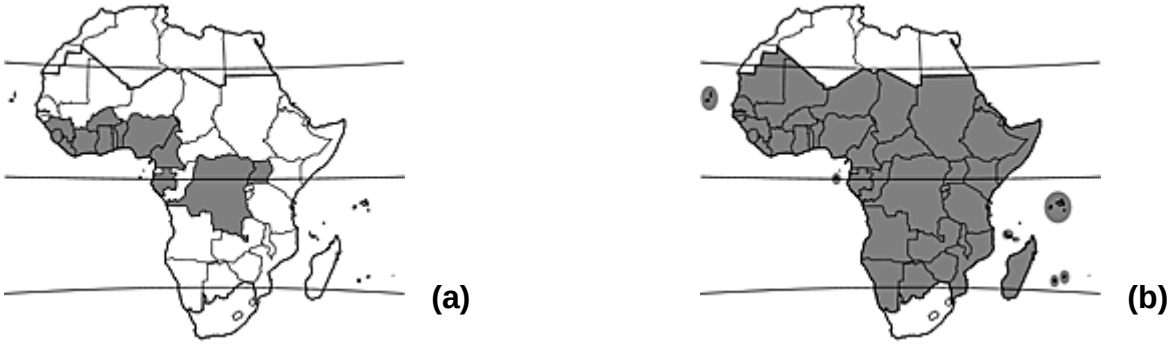


Figure 1: distribution of *Abelmoschus caillei* (a) and *Abelmoschus esculentus* (b) in Africa represented by grey colour

Source: (Siemonsma and Hamon, 2004; Siemonsma and Kouamé, 2004)

Ab. esculentus is widespread in tropical, subtropical and warm temperate regions, but is particularly popular in West Africa, India, the Philippines, Thailand and Brazil. Besides occurring throughout the distribution area of *Ab. caillei*, it has been reported from the whole of tropical Africa (Figure 1b) (Siemonsma and Kouamé, 2004). Most tropical cultivars of *Ab. esculentus* show quantitative short-day responses like on flowering affected by day length in the coastal areas of the Gulf of Guinea (5°N), but qualitative responses also occur more inland at higher latitudes (10°N) with very tall non-flowering plants. The shortest critical daylength reported is 12 hours 30 minutes (Siemonsma and Kouamé, 2004). *Ab. caillei* shows a qualitative short-day response even at latitude of 5°, the shortest critical day length reported being 12 hours 15 minutes. Even at this latitude, vegetative periods of 8 to 9 months occur when sown under the 'long-day' conditions of the rainy season. Apart from these qualitative responses, most local types show quantitative short-day responses. *Ab. caillei* is, therefore, not suitable for semi-arid

and arid regions beyond latitudes of 12°N and 12°S where the day length is over 12 hours. Okra in general tolerates a wide variety of soils but prefers well-drained sandy loams, with pH 6 to 7, and a high content of organic matter (Siemonsma and Hamon, 2004).

I.1.3. Growth and development of okra

There are only two species of okra grown in Cameroon: *Abelmoschus esculentus* and *Ab. caillei*. The former differs in several respects from the latter, but the epicalyx offers the best discriminating characteristic: the width of the epicalyx segments is 4 to 13 mm in *Ab. caillei* and 0.5 to 3 mm in *Ab. esculentus* (Figure 2).

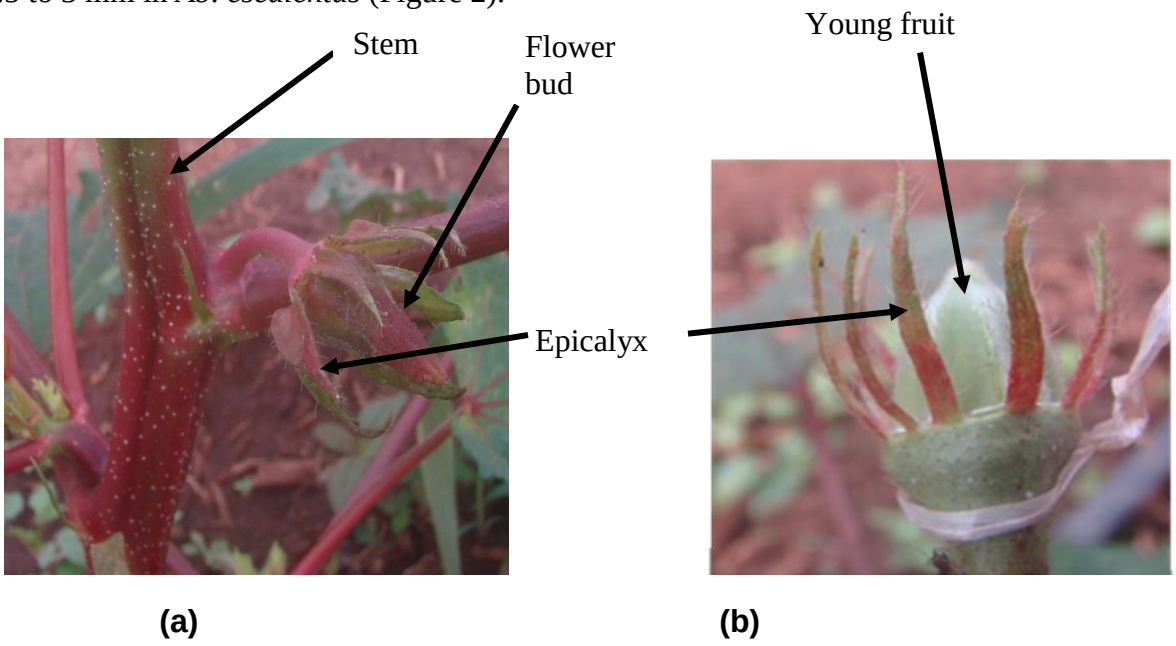


Figure 2: Epicalyx of *Ab. caillei* (a) and *Ab. esculentus* (b)
Source: AVRDC.

These two okra species can be quite reliably recognized based on fruit form, but not with absolute certainty. Fruits of *Ab. caillei* are ovoid, whereas those of *Ab. esculentus* are cylindrical to pyramidal. Information related to *Ab. caillei* has often been attributed to *Ab. esculentus* and/or *Ab. manihot*, thus literature has to be interpreted with care. *Ab. manihot* differs from *Ab. caillei* by a smaller number of epicalyx segments (4 to 8), and much smaller fruits (3.5 to 6 cm long) which are inedible because they are covered with prickly hairs (Siemonsma and Hamon, 2004; Siemonsma and Kouamé, 2004).

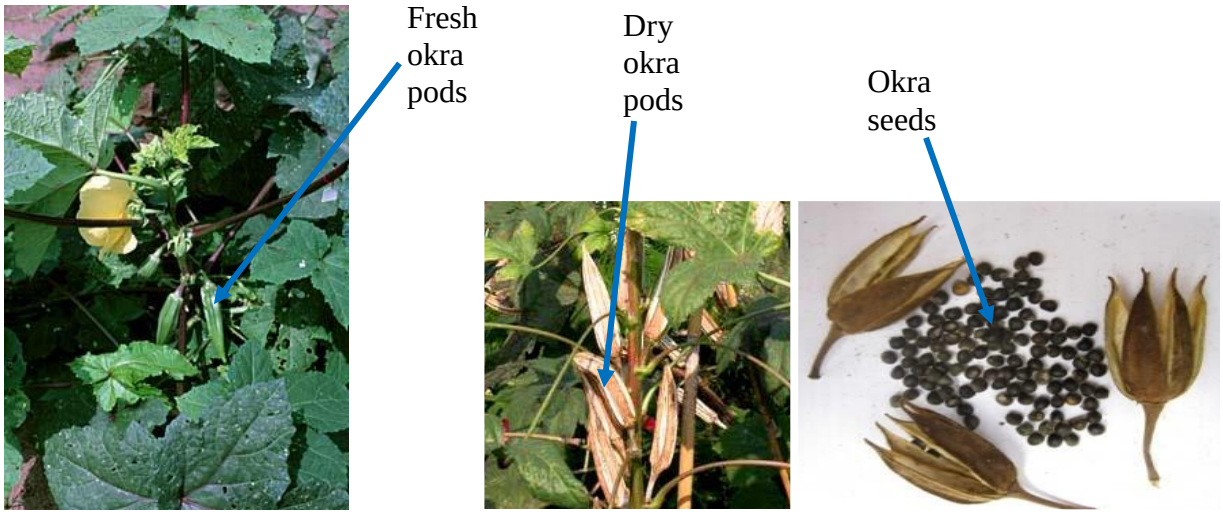
Okra is a stout, annual to biennial, erect herb up to 4 m tall with a crop cycle that varies according to location. *Ab. caillei* flowers within 50 to 110 days after sowing (sowing in October: short-days) and within 65 to 270 days after sowing (sowing in March: long-days). Short-day types, planted at the beginning of the rains (March) do not flower by the end of the rainy season (November), but are so well developed vegetatively that they easily survive the dry season without supplementary water, and bear fruit in a period of scarcity. This explains why in African languages, West African okra is sometimes referred to as 'late okra' or 'dry-season okra'. Crop duration thus shows enormous variation depending on cultivar, locality and season, and varies from 4 months to 12 months. Comparing cultivars of similar earliness, West African okra has a considerably longer productive period making it suitable for home gardening (Siemonsma and Hamon, 2004).

Common okra, *Ab. esculentus* flowers within 45 to 80 days after sowing (sowing in October: short-days) and within 55 to 105 days after sowing (sowing in March: long-days). Crop duration rarely attains six months (Siemonsma and Kouamé, 2004).

I.1.4. Importance of okra

Okra is called by different names in various parts of the world. It is named as lady's finger in England, gumbo in USA, guino-gombo in Spanish, guibeiro in Portuguese, and bhindi in India (Chauhan, 1972), krajiab kheaw in Thailand, okra plant, ochro, okoro, quimgombo, quingumbo, gombo, kopi arab, kacang bendi and bhindi in South East Asia. However, in Middle East it is known as bamia, bamya or bamieh (Ndunguru and Rajabu, 2004). It is also named quimbombo in Cuba, gombo commun, gombo, gumbo in France, mbamia and mbinda in Sweden, and in Japan as okura (Chauhan, 1972; Lamont, 1999). Lastly, it is also found in Taiwan, where it is described as qiu kui (Siemonsma and Kouame 2000). In West and Central Africa (WCA), it is Gombo (French), Miyan-gro (Hausa), La (Djerma), Layre (Fulani), Gan (Bambara), Kandia (Manding), Nkruma (Akan), Fetri (Ewe) (Kumar *et al*., 2010) and bikoye in Bassa.

In Cameroon, the young tender fruits (figure 3a) of okra are usually cut into small pieces for use fresh as paste or dried and ground as powder, then added to sauces to be served with a variety of starchy foods such as ''Achu'' from *Colocasia* sp. macabo, ''fufu'' from millet, sorghum, rice, corn, cassava and garri. The young leaves are also cooked and eaten (George, 1989).



(a) (Siemonsma and Hamon, 2004) (b) (Sukprakarn *et al.*,2006) (c) (Nguelieu, 2009)

Figure 3: okra plants with fresh pods (a), and dry pods (b) dry seeds (c)

Extracts from the seeds can be an alternative source for edible oil. The greenish yellow edible oil has a pleasant taste and odour, and is high in unsaturated fats. The oil content of the seed is quite high at about 40%. Potassium, sodium, magnesium and calcium are the principal elements in pods of okra, which contain about 17% seeds (Table 1). The presence of iron, zinc, manganese and nickel has also been reported (Moyin-Jesu, 2007). Fresh okra pods are low in calories (20 kcal per 100 g), no fat, high in fibres, and have several valuable nutrients, including about 30% of the recommended levels of vitamin C (16 to 29 mg), 10% to 20% of folate (46 to 88 mg) and about 5% of vitamin A (14 to 20 RAE (retinol activity equivalents) (NAP, 2006). Both pod skin (mesocarp) and seeds are excellent sources of zinc (80 mg/g) (Cook *et al*., 2000). Okra seed is mainly composed of oligomeric catechins (2.5 mg/g of seeds) and flavonol derivatives (3.4 mg/g of seeds), while the mesocarp is mainly composed of hydroxycinnamic and quercetin derivatives (0.2 and 0.3 mg/g of skins). Pods and seeds are rich in phenolic compounds with important biological properties like quartering derivatives, catechin oligomers and hydroxycinnamic derivatives (Arapitsas, 2008). These properties, along with the high content of carbohydrates, proteins, glyco-protein, and other dietary elements (Table 1) enhance its importance in the human diet (Manach *et al*., 2005; Asawalam *et al*., 2007; Arapitsas, 2008). Dried okra sauce does not provide any beta-carotene (vitamin A) or retinol (Avallone *et al*., 2008). Fresh pods are the most important source of viscous fibre, an important dietary component

to lower cholesterol (Kendall and Jenkins, 2004) and the viscosity eases consumption of starchy foods (Schippers, 2000).

Table I: composition of okra fruits and leaves per 100 g edible portion (Leung *et al.*, 1968)

Nutrients	Fruit	Leaf
Water	88.6 g (85.7 to 90.2)	81.5 g (75.3 to 92.4)
Energy	144 kJ (36 kcal)	235 kJ (56 kcal)
Protein	2.1 g (1.1 to 3.0)	4.4 g (2.8 to 5.6)
Fat	0.2 g	0.6 g
Carbohydrate	8.2 g	11.3 g
Fibre	1.7 g	2.1 g
Calcium (Ca)	84 mg (55 to 142)	532 mg (258 to 635)
Phosphorus (P)	90 mg	70 mg
Iron (Fe)	1.2 mg (1.1 to 1.5)	0.7 mg
β-carotene	185 µg (180 to 190)	385 µg
Thiamin	0.04 mg	0.25 mg
Riboflavin	0.08 mg	2.8 mg
Niacin	0.6 mg	0.2 mg
Ascorbic acid	47 mg (20 to 126)	59 mg (9 to 75)

Compared to other fleshy fruit-vegetables (tomato, eggplant), okra is particularly rich in calcium and ascorbic acid. Carbohydrates are mainly present in the form of mucilage; that of the young fruits of *Abelmoschus esculentus* is made up of sugar units and amino acids. The main components are galactose (25%), rhamnose (22%), galacturonic acid (27%) and amino acids (11%). Its seeds contain about 20% proteins and 20% oil (Agbo *et al.*, 2008) and is ranked first in terms of calories (4550 kcal/kg) among all vegetable crops and overall fourth of all foods and drinks (Babatunde *et al.*, 2007).

Mucilage is suitable for medicinal and industrial applications; it has a medical application as a plasma replacement or blood volume expander, and is useful against genito-urinary disorders, spermatorrhoea and chronic dysentery (Nandkarni, 1927). It has also been reported in curing ulcers and relief from haemorrhoids (Adams, 1975). The work of Vayssade *et al*. (2010) on anti-proliferative and proapoptotic actions of pectin on B16F10 melanoma cells might open the way to new melanoma therapies. Tseng *et al,* (2004) showed a reduction in prostate cancer risk in Southern American feeding pattern characterized by foods such as cornbread, grits, sweet potatoes, okra, beans, and rice. Monte *et al.* (2014) demonstrated that lectin of *Ab. esculentus* promotes selective antitumor effects in human breast cancer cells. Industrially, mature fruits and

stems containing crude fibre are used in the paper industry. Ripe seeds are roasted, ground and used as a substitute for coffee (Markose and Peter, 1990). The roots and stems are used for cleaning the cane juice from which Gur or brown sugar is prepared (Chauhan, 1972). Okra is also used as a good source of gum and its fibres are traditionally used to make rope. The ground pulp of *Ab. caillei* stem is used as a stabilizer when making Pita beer in northern Ghana (Schippers, 2000).

I.1.5. Production statistics

The crop is a widely cultivated vegetable and can be found in almost every market in Africa. In Ghana, it is the fourth most popular vegetable after tomato, pepper and eggplant. In Sudan, the common okra is the third or fourth most popular vegetable. In Cameroon, the two species *Abelmoschus caillei* and *Abelmoschus esculentus* combined represent the second most important vegetable in the market after tomato (Schippers, 2000 and 2002). In terms of number of producers, okra is the fourth most important vegetable crop cultivated in Cameroon after tomato, pepper and huckleberry (Abang *et al*., 2013), and among the top five in Africa (Ellis-Jones *et al.*, 2008). Nigeria is the largest producer (1.04 mt) in Africa while Sudan (Former) follows with only 250000 tonnes. Cameroon occupies the fifth and eight positions in African and the world respectively with only 72661 tonnes (0.84% of world production) produced in 2013 (FAOSTAT, 2015). Worldwide production is estimated at 8.69 million tonnes annually. India is the highest producer in the world with 6.4 million tonnes (73%) produced from over 0.35 million ha land, followed by Nigeria (13 %). West Africa accounts for 76% of total production in Africa (FAOSTAT, 2015) (Figure 4). West African okra also known as Guinean type accounts for only 5% of the world production and the remaining is common okra 95%. It is only in West and Central Africa (accounting for about 10% of world production) that two species are both used. They share the market roughly fifty-fifty in this region (Siemonsma and Hamon, 2004; Siemonsma and Kouame, 2004).

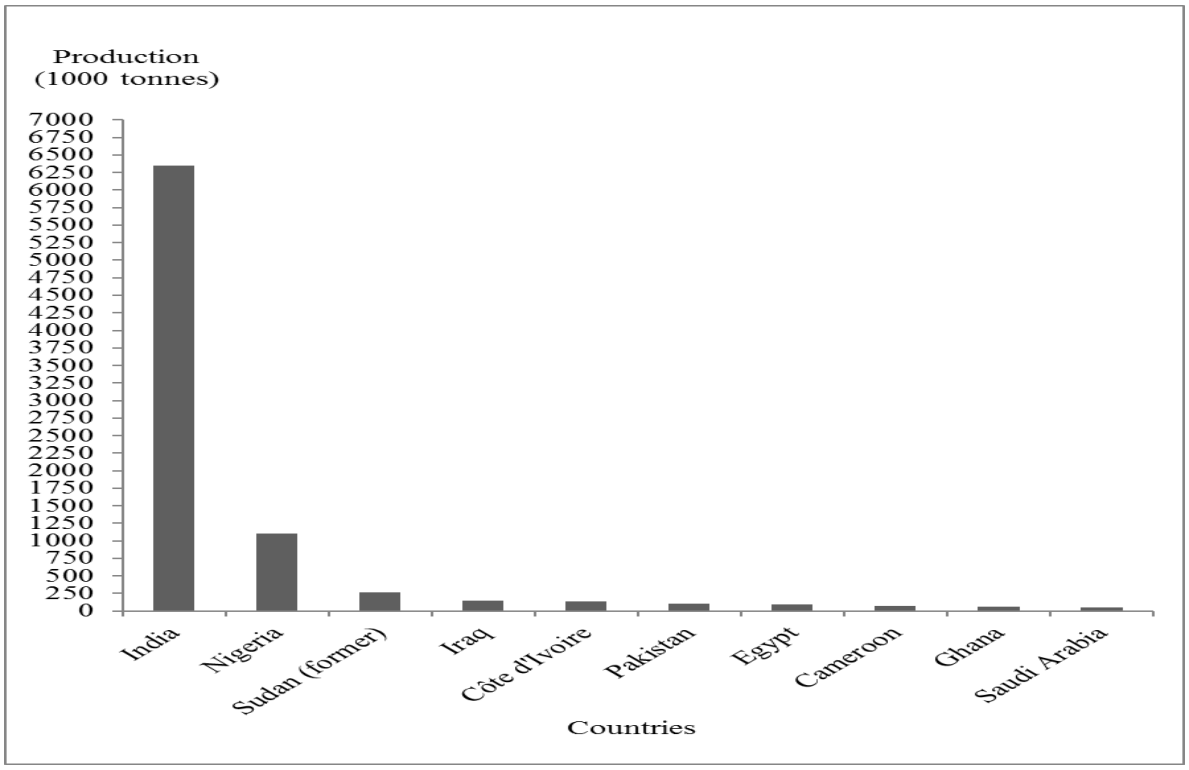


Figure 4: annual production of okra for the top 10 countries in the world (FAOSTAT 2015).

I.2. Aphids as pests of okra

I.2.1. What are aphids?

Aphids are pear-shaped 1 to 2 mm long soft-bodied insects with long legs, antennae, long slender mouthparts which they use to suck sap and often feed in dense groups. Species are difficult to distinguish from one another, but have similar management methods. They vary in colour depending on species and host plants. Some are waxy due to a waxy white secretion over their body. Most species have a pair cornicle projecting to the posterior end, which distinguishes aphids from all other insects. Adult are wingless, but also occur in winged forms, when populations are high or during spring and fall. The ability to produce winged forms provides them

with a way to disperse to other plants. Unlike leafhoppers, plant bugs and other insects that might be confused with them, aphids do not move rapidly when disturbed (Flint, 2013).

I.2.2. Aphid species recorded on okra

There are more than 4700 species of Aphididae in the world (Remaudiere and Remaudiere, 1997). About 450 species of them are recorded on crops, and 100 species being of economic importance (Blackman and Eastop, 2000). Aphid species recorded on okra are *Aphis craccivora* Koch (Himiptera; Aphididae), *A. gossypii* Glover (Himiptera; Aphididae), *A. spiraecola* Patch (Himiptera; Aphididae), *A. albella* Nevsky (Himiptera; Aphididae), *Myzus persicae* Sulzer (Himiptera; Aphididae), *Aulacorthum solani* Kaltenbach (Himiptera; Aphididae), *Smynthurodes betae* Westwood (Himiptera; Aphididae), *Macrosiphum euphorbiae* Thomas (Himiptera; Aphididae) and *Toxoptera odinae* van der Goot (Himiptera; Aphididae) (Blackman and Eastop, 2000). However, their pest status on okra, except *A. gossypii,* has not been reported. *A. craccivora* has a marked preference for Leguminosae; therefore, it is called the black legume aphid and the cowpea aphid or the groundnut aphid. *A. gossypii* is more polyphagous than *A. craccivora;* it attacks a wide range of plants including cotton, cucurbits, citrus, coffee, cocoa, eggplant, peppers, potato, okra, hibiscus and many ornamentals (Blackman and Eastop, 2000). This insect is a major pest of cucurbits and cotton, another Malvaceous crop like okra. *A. spiraecola* (Patch) is also highly polyphagous but infests mostly plants of the families of Caprifoliaceae, Compositae, Rosaceae, Rubiaceae and Rutaceae. It is also called the spiraea aphid or citrus aphid, the citrus being the most important host crop (Blackman and Eastop, 2000). The primary host of the green peach aphid, *Myzus persicae,* is *Prunus persica* var. Nectarine, but it also infests other plants of the genus *Prunus* such as *P. nigra, P. tenella* and *P. serotina*. *Aulacorthum solani* is the Foxglove aphid, also called the glasshouse-potato aphid; it is extremely polypghagous to both monocots and dicots but not Gramineae; it is common on potatoes and soybean in Korea and Japan (Blackman and Eastop, 2000). The bean root aphid, *Smynthurodes betae,* has *Pistacia atlantica* and *Pistacia mutica* as primary hosts. The secondary hosts are *Artemisia* spp. and *Arctium* spp. (Compositae), *Phaseolus* spp*, Vicia* spp., and *Trifolium* spp (Leguminosae), *Solanum tuberosum, Solanum nigrum, Solanum lycopersicum* (Solanaceae), and sometimes species of the genera *Beta, Brassica, Capsella, Gossypium, Heliotropum, Rumex*, etc. *Smynthurodes betae* are rarely found on monocots (Gramineae and Cyperaceae). *Macrosiphum*

euphorbiae primarily infests *Rosa* spp., but it is polyphagous and particularly favoured by the secondary host *Solanum tuberosum.* No primary host has been indicated for *Toxoptera odinae*; rather it is said to be polyphagous on tropical shrubs, especially the families Anacardiaceae (*Anacardium* spp., *Mangifera* spp. and *Rhus* spp.), Araliaceae (*Aralia, Polyscias* and *Kalopanax* genera), Caprifoliaceae (*Viburnum* spp.), Ericaceae (*Rhododendron* spp.), Pittosporaceae (*Pittosporum* spp.), Rubiaceae (*Coffea* spp, *Mussaenda* spp) and Rutaceae (*Citrus* spp.). *A. albella* is not well known (Blackman and Eastop, 2000); thus, *A. gossypii* is the only species that require attention on okra now.

Eighteen most polyphagous aphid species include the black bean aphid (*A. fabae* Scopoli), buckthorn–potato aphid (*A. nasturtii* Kaltenbach), cucumber tree aphid (*Aulacorthum magnoliae* Essig and Kuwana), leaf-curling plum aphid (*Brachycaudus helichrysi* Kaltenbach), shallot aphid (*Myzus ascalonicus* Doncaster), *Myzus cymbalariae* Stroyan, the ornate or violet aphid (*Myzus Ornatus* Laing), *Sinomegoura citricola* van der Goot, brown Citrus Aphid (*Toxoptera aurantii* Boyer de Fonscolombe), black citrus or oriental citrus aphid (*Toxoptera citricidus* Kirkaldy). These species include eight of the nine aphid species recorded on okra and are presented in Figure 5.

Macrosiphum euphorbiae

Brachycaudus helichrysi

Smynthurodes betae

A. nasturtii

Aphis spiraecola

A. craccivora

Aphis fabae

Aphis gossypii

Aulacorthum magnoliae

Aulacorthum solani

Myzus ascalonicus

M. cymbalariae

Myzus ornatus

Myzus persicae

Sinomegoura citricola

Toxoptera aurantii

Toxoptera citricida

Toxoptera odinae

Figure 5: the most polyphagous aphid species (compiled from aphid.aphidnet.org).

I.3. The cotton Aphid: *Aphis gossypii* Glover (Hemiptera: Aphididae)

I.3.1. Taxonomy

Aphis gossypii Glover (Hemiptera: Aphididae) was first described in 1876 by Glover (Palmer, 1952). Vilcinskas (2016) adopted the following taxonomy for this insect (Table II).

Table II: taxonomic hierarchy

Kingdom	Animalia (Eumetazoa)
Subkingdom	Bilateria
Infrakingdom	Protostomia
Superphylum	Ecdysozoa
Phylum	Arthropoda

Subphylum	Hexapoda
Class	Insecta
Subclass	Pterygota
Infraclass	Neoptera
Superorder	Paraneoptera
Order	Hemiptera
Suborder	Sternorrhyncha
Superfamily	Aphidoidea
Family	Aphididae
Genus	*Aphis* Linnaeus, 1758
Species	*Aphis gossypii* Glover, 1876

I.3.2. Identification of *Aphis gossypii* from other species recorded on okra

The following identification has been proposed by Stoetzel *et al.* (1996). The cotton aphid, *Aphis gossypii,* is a small to medium sized organism. Apterae are very variable in colour and large specimens are dark green, almost black, but adults produced in crowded colonies at high temperature may be less than 1 mm long and pale yellow to almost white. The nymphs are mostly light green mottled to dark green, with dark Siphunculi and a pale or dusky Cauda. Antennal segments I, II, apical half of Processus Terminalis and area around the primary Rhinarium of VI are dark, and remainder of antennae pale. Cauda apically broadly rounded, often with 4 to 7 hairs. Cauda is dusky but lighter than Siphunculi. Abdominal Dorsum is without any pigmentation. The adult of the reference aphid species, *Aphis craccivora,* is always shiny black. Its immature stages are lightly dusted with wax and light brownish, the Siphunculi and Cauda are black. Abdomen black dorsally extending laterally except in small specimens; Antenna 2/3 as long as body; segment I, II, and apex of V dark, segments III, IV and basal V pale; Cauda with 4 to 7 hairs or setae. Other aphids are similar in appearance to *A. gossypii*. One other species commonly encountered and confused with the cotton aphid is *A. spiraecola* Patch, the spirea aphid. In *A. gossypii,* the cauda is pale to dusky and has two or three pairs of setae. In *Aphis spiraecola,* the cauda is dark brown to black and has five or six pairs of setae. Stoetzel *et al.* (1996) established the following key for identification of *A. gossypii* (Figure 6 to 8). The features used in the identification of aphids are the length of terminal processus and base, presence of cornicles or siphunculi, cauda structure, antennal tubercles development, number of antennae segments, terminal process structure, cauda length with respect to that of cornicles, cauda pale or dusky and number of pairs of setae, patches or bands on dorsum of abdomen,

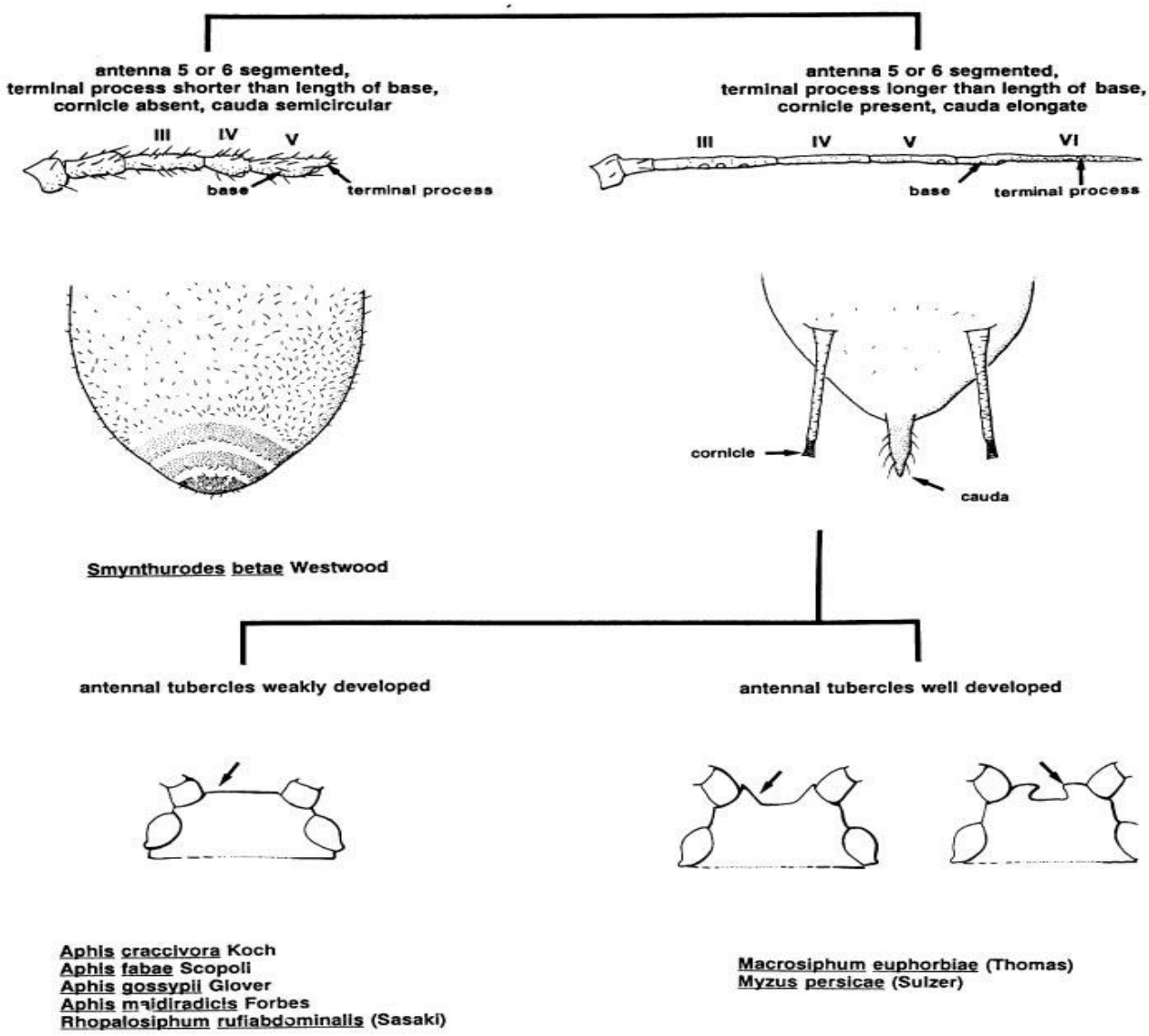


Figure 6: general identification of *Aphis gossypii* (Stoetzel *et al.*,1996).

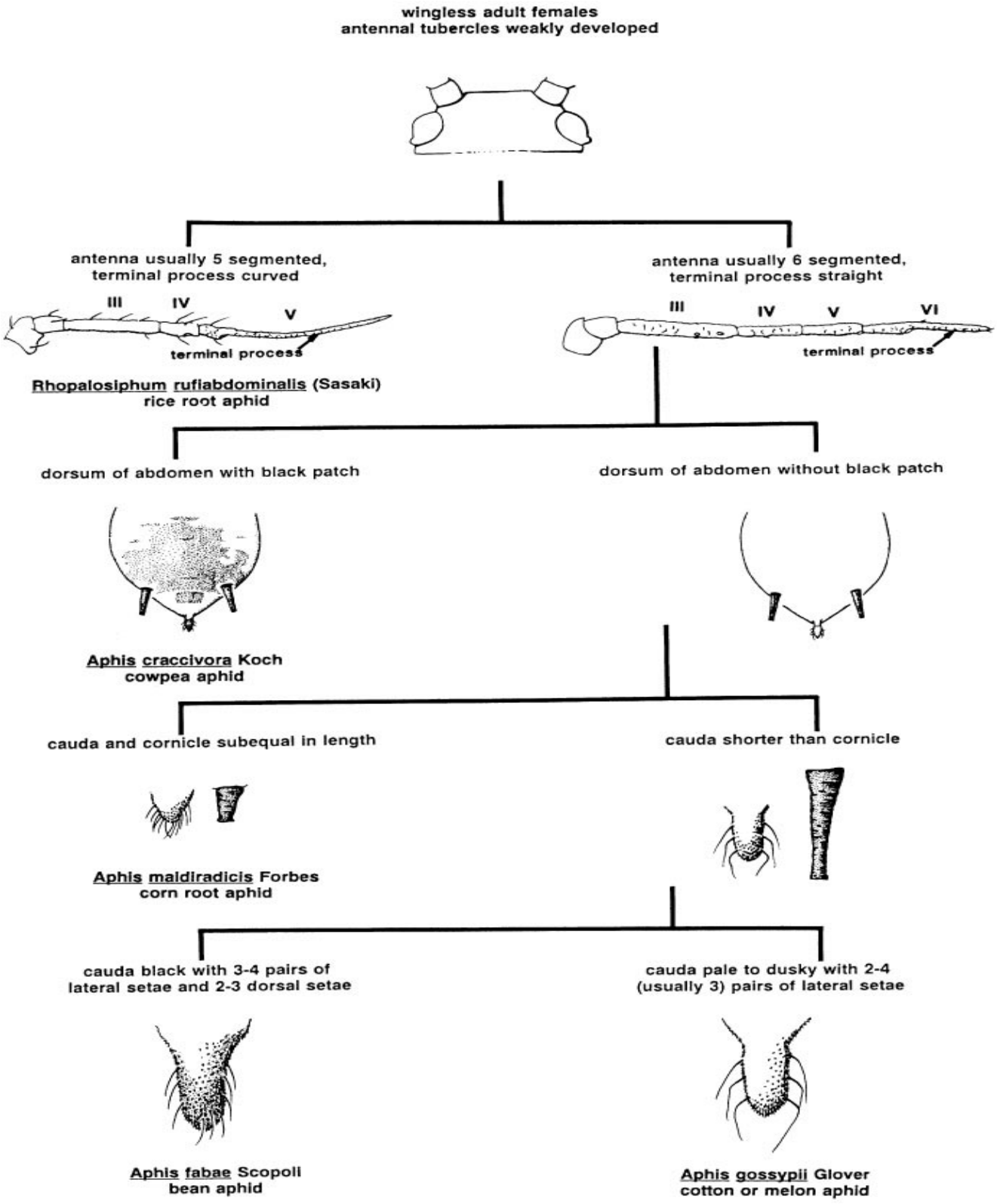


Figure 7: identification of wingless *Aphis gossypii* (Stoetzel *et al.*, 1996).

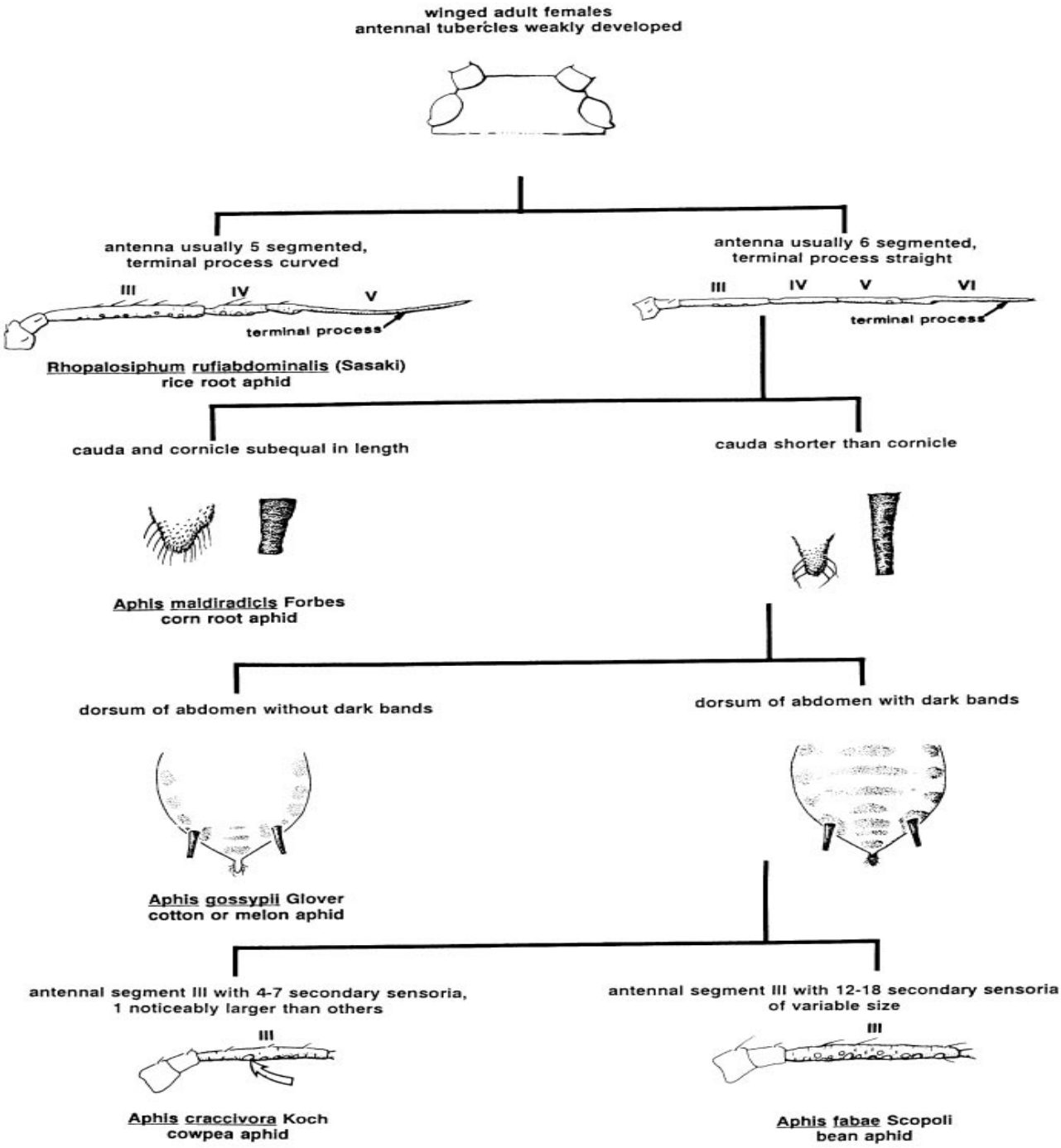


Figure 8: identification of winged *Aphis gossypii* (Stoetzel *et al.*, 1996).

I.3.3. Origin of *Aphis gossypii*

Short hairs on legs and antennae, a cauda that is usually paler than the siphunculi and bears rather few hairs, make it easy to apply the name *Aphis gossypii* to aphids collected on crops or other non-indigenous plants anywhere in the world. This is, however, an over simplification of

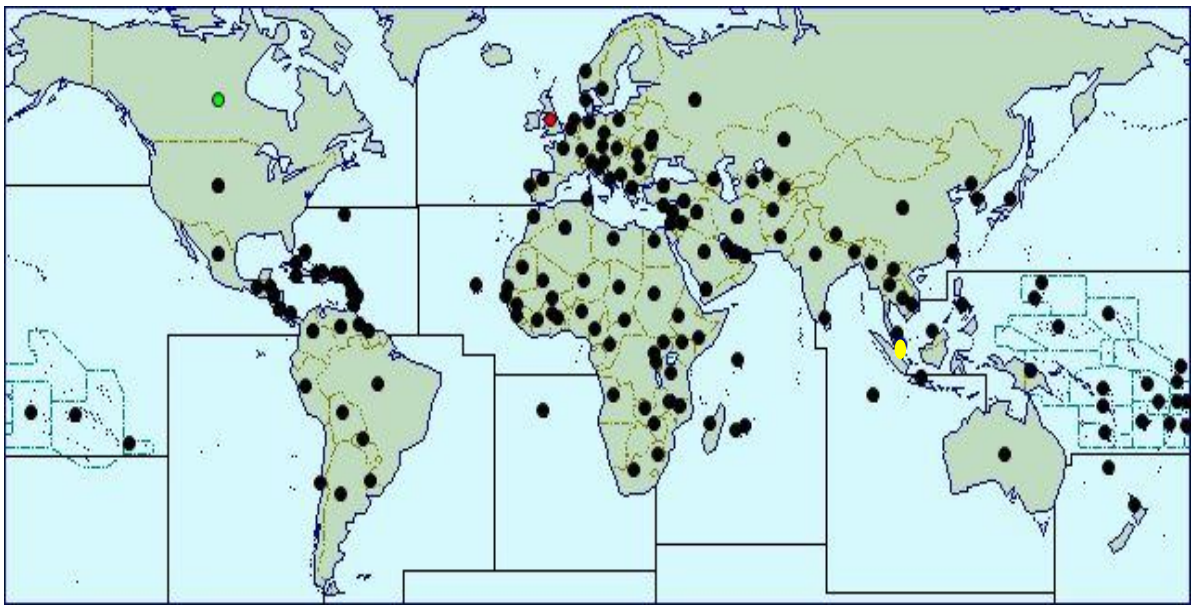
the taxonomic problem, especially when one compares accounts of *A. gossypii* in Europe and East Asia. In Europe, *A. gossypii* is classed as a subspecies in the *Aphis frangulae* complex, a group of closely related and morphologically almost indistinguishable indigenous species that use buckthorn, *Frangula alnus*, as their primary host (Stroyan, 1984; Heie, 1986). *A. gossypii* was regarded as the only member of the group that does not have a sexual phase on buckthorn, overwintering parthenogenetically in Northern Europe (Blackman and Eastop, 2007). From these characteristics, it was shown that this pest originated in Europe as a permanently parthenogenetic, highly polyphagous and adaptable offshoot of the *A. frangulae* complex, and spread from there to all parts of the world. However, such a conclusion is difficult or impossible to reconcile since the same species in Japan and China produces parthenogenetic generations and is equally polyphagous, but there is an annual sexual phase. Overwintering as eggs occurs in East Asia on different plants species, including *Frangula* spp., *Hibiscus syriacus*, *Celastrus orbiculatus* and *Rubia cordifolia* (Inaizumi, 1980; Zhang and Zhong, 1990). It is possible that some of these populations have diverged as a result of differential selection among these primary hosts; populations overwintering on *R. cordifolia* in Japan, for example, seem to be isolated from those on other primary hosts, and are possibly a separate taxon (Inaizumi, 1981).

Earlier, Kring (1959) had demonstrated that populations in Connecticut, USA, also had a sexual phase, using *H. syriacus* and *Catalpa bignonioides* as primary hosts. Therefore, the origin of *A. gossypii* could be in Europe, East Asia, or North America. However, North America is unlikely to be its origin, because there is no indigenous North American *Aphis* species of the group of those closely related to *A. gossypii* that use *Frangula* spp. as primary hosts (Blackman and Eastop, 2007). In East Asia, there are indigenous species related to *A. gossypii* that have a sexual phase on *Frangula* spp. such as the soybean aphid *A. glycines*, but none of these seem quite so similar in morphology to *A. gossypii* as the European *A. frangulae* group. Blackman and Eastop (2007) therefore suggested extensive work encompassing the entire geographical and host-plant range of *A. gossypii* and including comparisons with related species in Europe and East Asia.

I.3.4. World distribution of *Aphis gossypii*

The cotton aphid occurs in tropical and temperate regions (Capinera, 2005). This insect is completely cosmopolitan, absent only in northern parts of Canada, Europe and Asia (Figure 9).

The first reported occurrence of *Aphis gossypii* was on Oahu in 1909; this insect is now present on all islands throughout the USA (Kessing and Mau, 2007). It has two common names recognized by the Entomological Society of America: the cotton aphid and melon aphid. The cotton aphid was first found to be a serious pest of cotton in 1854 in South Carolina, but was recognized as a pest many years before that (Slosser *et al*., 1989). Texas' melon industry first reported this aphid as a pest in 1892; the cotton industry soon followed in 1916 having the same pest problem (Paddock, 1919). Because melon aphid sometimes overwinters in greenhouses, and may be introduced into the field with transplants in spring (temparate zones) or beginning of rainy season (tropics), it has potential to be damaging almost anywhere.



=Presence, =Widespread, =Localised, =Check regional map for distribution within the country

Figure 9: world distribution of *Aphis gossypii* (http://www.cabi.org/isc/datasheet/6204).

I.3.5. Reproduction, growth and development of *Aphis gossypii*

In parts of the tropical regions where winter is mild, there is no need for an overwintering egg stage. Reproduction does not involve mating and laying of eggs. Females give birth to live female nymphs; because of this type of reproduction, populations are composed solely of females. There are many generations of this aphid throughout the year. The life cycle differs greatly between the temperate and the tropical regions. In the north or temperate regions, this aphid over-winters as eggs. Female nymphs hatch from eggs laid on backs of trees in the spring.

They feed, develop, and reproduce parthenogenetically or viviparously throughout summer. Winged females may also be produced, that disperses to other hosts and form new colonies (Capinera, 2005). The dispersants feed and may produce both winged and wingless female offspring (Figure 10). Under high population density, deterioration of the host plant or upon arrival of autumn, production of winged forms predominates. During periods unfavourable for the host plant, small yellow or white forms of the aphid are also produced irrespective of the region (Figure 10). Late in autumn, winged females identify primary hosts, and both males and egg-laying (oviparous) females are produced. Mating occurs and females oviposit: eggs are the only form for overwintering or dormancy under cold conditions. Under warm conditions, a generation can be completed parthenogenetically in about seven days (Capinera, 2005).

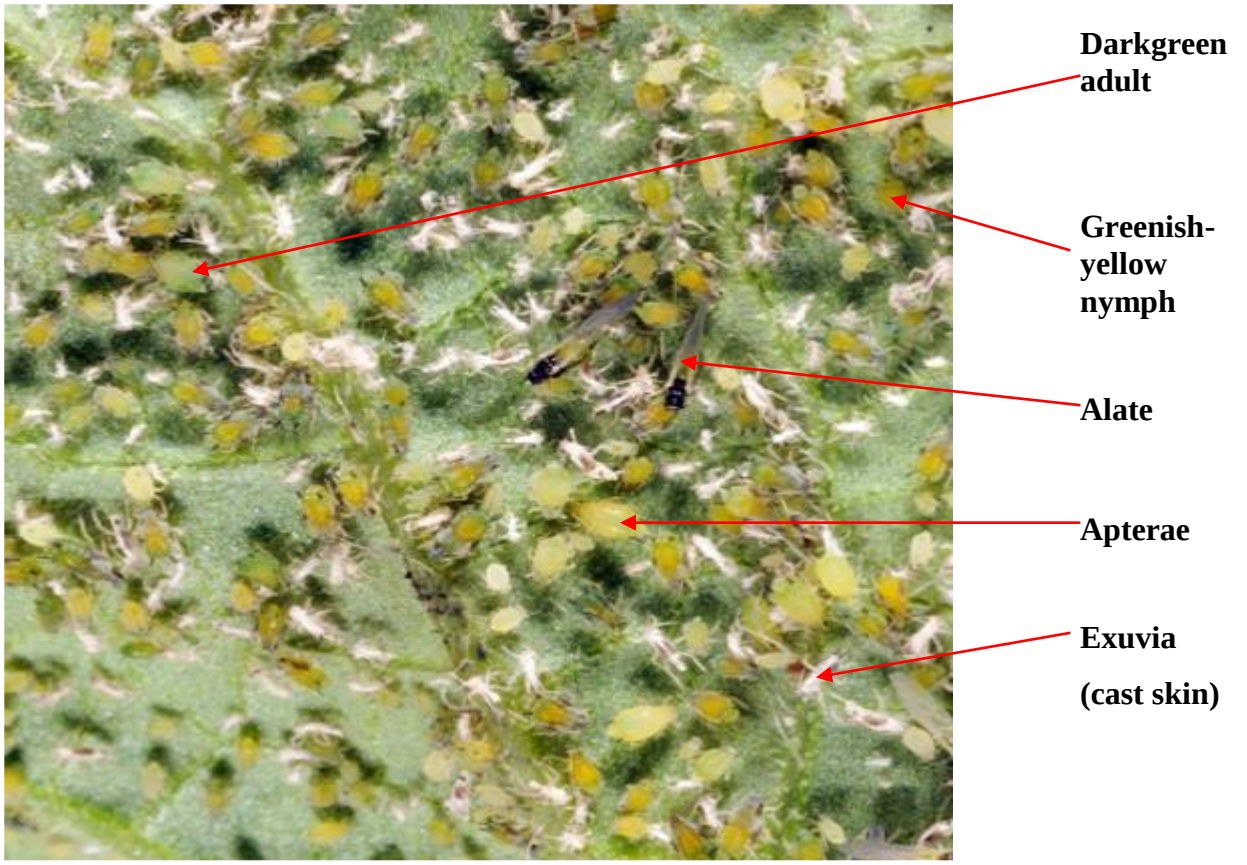


Figure 10: *Aphis gossypii* on okra showing colour variation, alates and apterae (Foster R.E and Obermeyer J., 2017).

When the eggs are first laid, they are usually yellow, but soon become black and shiny. As noted previously, the eggs normally are deposited on catalpa and rose of sharon. This insect has four nymphal stages separated by moults. Each stage lasts from 1to 3 days for a total nymphal period

of 4to12 days. Nymphs resemble adults, except for their smaller size. They do not have wings and they vary in colour from pale brown to gray or green and often have dark head, thorax and wing pads, and dark-green distal portion of the abdomen. The body is not shiny in colour because of the presence of waxy secretions (Kessing and Mau, 2007).

The wingless parthenogenetic females are 1 to 2 mm in length. Their body is also variable in colour. The most common is light green mottled with dark green, but whitish, yellow, pale green, and dark green forms sometimes appear. The legs are pale but the tips of the tibiae and tarsi are black; the cornicles are also black. Small yellow forms are produced in response to population increase or plant stress. Winged parthenogenetic females measure 1.1 to 1.7 mm in length. The wing veins are brown. Male and oviparous female are dark purplish green. The duration of the adult's reproductive period varies as a function of temperature (Figure 11) but generally about 15 days, and the post-reproductive period is five days. Adults are smaller and paler in high temperatures. The optimal temperature for reproduction is reported to be about 21 to 27°C. Viviparous females produce about 70 to 80 offsprings at a rate of 4.3 per day (Capinera, 2005).

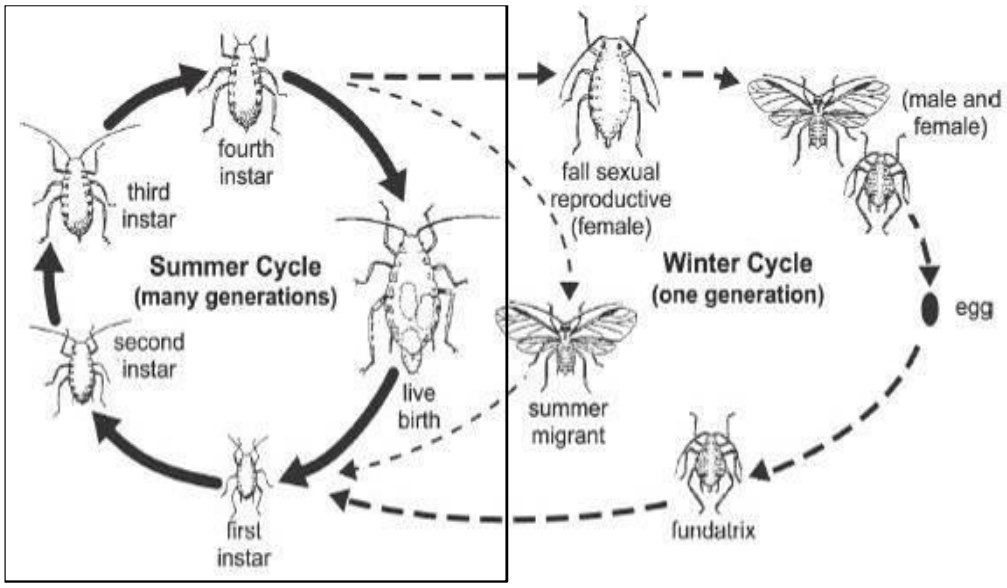


Figure 11: general life cycle of aphids showing asexual (summer cycle) and sexual (winter cycle) (Flint, 2013).

I.3.6. Host plants of *Aphis gossypii*

Aphis gossypii has a very wide host range. Although the taxonomy of its host plants is uncertain, it is estimated that 700 host plants exist worldwide. Among Cucurbitaceae, it can be a serious pest on watermelons, cucumbers, and cantaloupes, and to a lesser extent squash and pumpkin; this is the basis for the common name "melon aphid." In the south, cotton is an important host, which also explains the second common name, "cotton aphid" (Capinera, 2005). Economically important host plants include asparagus, avocado, banana, burdock, Chinese wax gourd, cucumber, edible gourds, eggplant, flowering ginger, green bean, guava, hibiscus, hyotan, luffa, orchid, papaya, peppers, potato, protea, pumpkin, spinach, taro, tomato, watermelon, zucchini, cantaloupes, squash pumpkin, cotton, bean, beet, crucifers, citrus, coffee, cocoa and okra (Leclant and Deguine, 1994; Kessing and Mau, 2007). Important weed hosts include Lamb's quarters, Shepherd's purse, Malva and Bidens (Kessing and Mau, 2007). Catalpa (*Catalpa bignonioides*), and rose of sharon (*Hibiscus syriacus*) were the primary hosts in northern parts of the globe. In the south, eggs are not commonly produced but primary or overwintering hosts are more numerous such as dock (*Rumex crispus* and *Lamium amphlexicaule*), boneset (*Eupatorium petaloiduem*) and citrus (*Citrus* spp.). Several researchers have noted the existence of host races (Moursi *et al.,* 1985; Guldemond *et al.,* 1994). Aphids reared on cotton could be transferred successfully to okra but not to cucurbits. Several authors using other combinations (Wang *et al*., 2004; Capinera, 2005) have subsequently showed this host specificity in races hosts. The infestation process begins as winged adult aphids come in from hideouts (usually the craggy bark of nearby trees) during early spring or around March, when weather is warm enough to allow their flight and migration. Humans can also introduce aphids on their bodies and on purchased plants. The insects can travel from one plant to another. In outdoor gardens, ants that feed on aphid honeydew often tend aphid colonies. Ants have been observed transporting aphids to new plants, therefore it has been hypothesized that the ants are "farming" aphids. Indoors, aphids can spread through flying or crawling.

I.3.7. Pest status of *Aphis gossypii* on okra

The undersides of leaves are preferred, but the entire host plant including buds, flowers and stems may be covered when populations are large (Figures 12).


(a) (Satyagopal and Singh, 2014)


(b) (Satyagopal and Singh, 2014)


(c) (Varela and Seif, 2004)

Figure 12: aphids on okra; a: lower leaf surface, b: young buds and c: okra fruit and flower.

Aphis gossypii sucks nutrients from the plant causing foliage to become chlorotic, and reduce fruit set. In addition, their feeding causes distortion and leaf curling downwards. Young plants may have reduced or stunted growth with deformed leaves (Figure 13). Moreover, this insect secretes honeydew, rich in sugars and amino acids, which provide a growing media for saprophytic fungi such as sooty mould (*Capnodium* spp., *Cladosporium* spp. and *Fumago* spp.) on plant tissues (Hillocks and Bretell, 1993). Sooty mould blackens the leaf and decreases photosynthetic activity (Elmer and Brawner, 1975). When found on the fruit, honeydew and sooty mould reduces its marketability. Ants and other insects, which may provide protection for the aphids from natural enemies, also feed on honeydew (Figure 14).


Figure 13: stunted okra plant with deformed leaves (Varela and Seif, 2004).


Figure 14: aphids/ants in symbiosis on okra (Doumbia and Seif, 2008).

I.4. Management of aphids

I.4.1. Cultural practices for the management of aphids

I.4.1.1. Timeliness of operation and cropping season

Kessing and Mau (2007) had demonstrated that dry weather conditions are favourable to aphids while heavy rainfall decreases their population sizes. Seasonality has also been shown to affect aphid incidence. Peak incidences have been noticed during first week of July in India for first season crop (March to July), and during first week of October for second season crop (Septembner to December) (Anitha and Nandihalli, 2008; Gulati, 2004; Hegde *et al.,* 2004). Late sowing of crops has been reported to lead to increased aphid infestation and *vice versa* (McGrath and Bale, 1990; McPherson *et al*., 1993).

I.4.1.2. Cropping systems

The wide host range of the cotton aphid makes crop rotation a difficult tactic to implement successfully. In addition, crops grown down-wind from infested fields are susceptible because aphids are weak fliers and tend to be blown about (Capinera, 2005). Plant should be destroyed immediately after harvest to prevent excessive dispersal, and it may be possible to destroy overwintering weed hosts. If continuous cropping is implicated with retention of aphid populations, then a crop-free period is needed (Capinera, 2005). Nderitu *et al*. (2008) evaluated the role of border-cropping systems and reported higher parasitism by *Aphidius* spp. and lower aphid infestation on okra field bordered by pigeon pea and maize compared with fallow area. The role of border cropping systems is to provide alternate hosts crops for natural enemies, repel the pest or act as an attractant or as a sink for insect pest from the main crop (Hooks and Fereres,

2006; Shelton and Badenes-Perez, 2006). However, the effectiveness of this control strategy will depend on the host status of the main crop as suggested by Kibaru (2004).

I.4.1.3. Soil fertilizer application

Fertilization is one of the most important factors that can influence the infestation by aphids (Cisneros and Godfrey, 2001; Slosser *et al.,* 2004). An excess of nitrogen (N) or deficiency of potassium (K) can lead to higher accumulation of amino acids then to higher attack rate by sucking insects (Jansson and Ekbom, 2002). The use of poultry manure as soil amendment reduces infestation by *Aphis gossypii* on okra plant (Baidoo and Mochiah, 2011). Even with high pest infestation on okra treated with compound fertilizers such as Nitrogen, Phosphorus and Potassium (NPK) or organic manure, the yield is not affected due to the phenomenon of tolerance (Baidoo and Mochiah, 2011). Godfrey *et al*. (2000) used a combination of managed nitrogen and water deficit to make conditions less favourable for aphids. Cisneros and Godfrey (1998) had demonstrated that aphid densities were three times higher under high applications of nitrogen fertilizer (218 kg/ha) than the low nitrogen input (55 kg/ha). A similar association was evident in work by Slosser *et al*. (1997). A range of nitrogen doses applied to cotton also revealed a consistent trend for greater aphid densities with high levels of nitrogen fertilizer (Godfrey *et al*., 2000). However, organic and synthetic fertilizers may differ in their effects on aphids.

I.4.1.4. Mulching

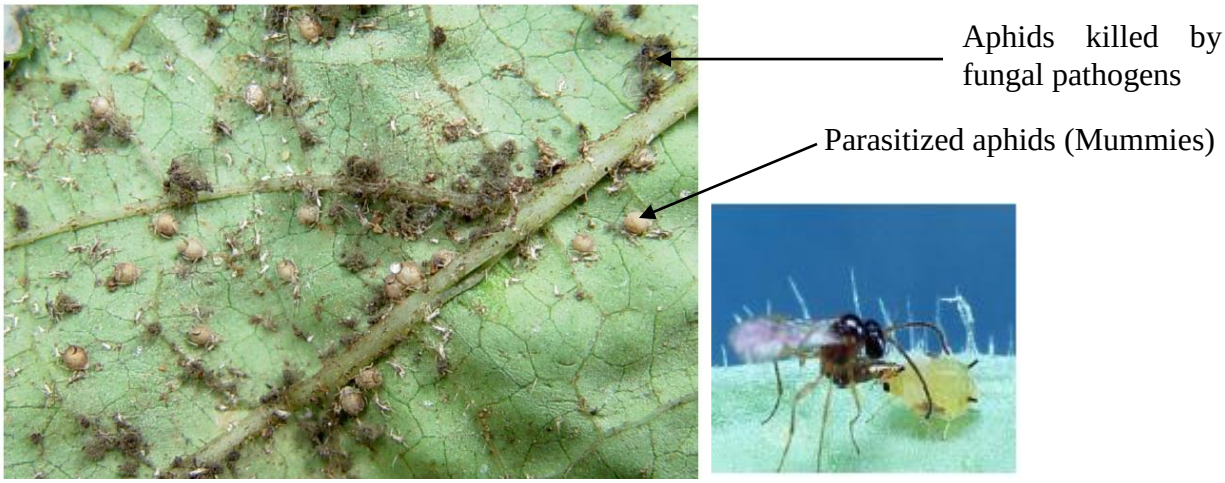
Row covers can be used to inhibit development of aphid populations. Several studies have investigated the optical properties of different mulches alongside their effect on aphid colonization and incidence of aphid-borne viruses (Greer and Dole, 2003; Jenni *et al*., 2003; Saucke and Doring, 2004 and Summers *et al*., 2004). These studies showed that transparent and aluminium-painted plastic mulches reduce population densities of aphids better than black or blue plastic mulches. It also reflected approximately four times as much UV light (<390 nm) than black or white mulches. Schmidt *et al.* (2004) found that straw led to lower aphid populations later in the season, and mulching was associated with higher spider populations. Floating row covers can reduce aphid density (Walters, 2003). They act by repelling alate aphids and preventing aphids at rest from inserting their stylets

I.4.1.5. Planting density and pruning

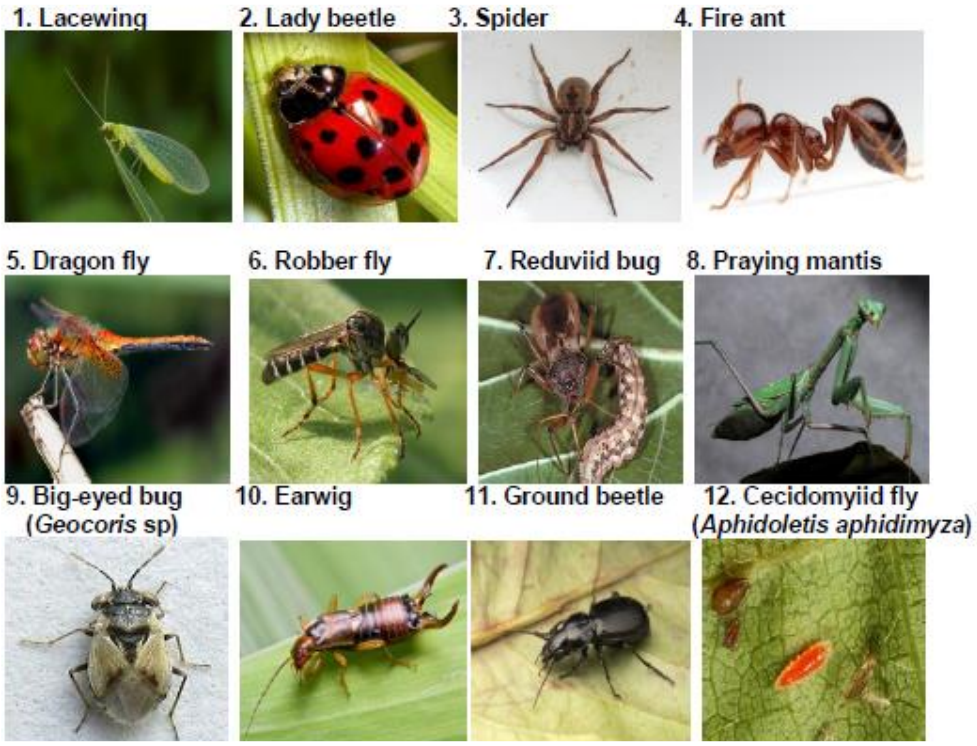
High densities of *Aphis craccivora* on cowpea in Uganda tended to be associated with low planting density (Karungi *et al*., 2000). The same pattern has been observed with *A. gossypii* in cotton crops in Texas, USA (Parajulee *et al*., 1999). Removal of terminal shoots of cotton using a pruning knife on seven sites in Cameroon had no effect at sites with low aphid densities, but did reduce aphid densities and the proportion of leaves infested. This method was considered inexpensive and well suited to local conditions (Wratten *et al*., 2007). Work in Central Africa also showed the effectiveness of hand removal of the terminal shoots of cotton plants at the end of the growing season (Deguine *et al*., 2000).

I.4.2. Natural enemies and biological control of aphids

Several beneficial insects help to control aphid populations through parasitism and predation (Figure 15). Parasitoids of *Aphis gossypii* found in Hawaii include *Aphelinus gossypi* (Timberlake) and *Lysiphlebus testaceipes* (Cresson). Some of the predators include *Chrysoperla* spp., *Nesomicromus vagus* (Perkins), *Zelus renardii* (Kolenati), *Platyomus lividgaster* (Mulsant), *Coelophora inaequalis* (Fabricus), *Allograpta obliqua* (Say) and *Leucopis nigricornis* (Egger). Coccinellid larvae, dults and syrphid larvae are voracious feeders of aphids (Kessing and Mau, 2007). Aphids can be parasitized by fungal pathogens or parasitoides (Figure 15 a). Satyagopal and Singh (2014) identified *Aphidius colemani* as the main parasitiod of aphids on okra and spiders, predatory bugs, beetles and parasitic wasps as major predators (Figure 15 b and c).



(a) (Varela and Seif, 2004) (b) (Satyagopal and Singh, 2014)



(c) (Satyagopal and Singh, 2014)

Figure 15: some natural enemies of aphids a: parasitized aphids and aphids killed by fungal pathogens, b: *Aphidius colemani* parasitizing an aphid c: some predators of aphids on okra.

van Driesche and Bellows (1996) classified biological control strategies into three main categories:

- classical, involving introduction of natural enemies into geographic areas where they did not previously occur;

- augmentation involving mass rearing and release of natural enemies that already exist in the system, but do not occur naturally in sufficient numbers;
- conservation biological control that includes the enhancement of naturally occurring populations of natural enemies, by means of habitat management or manipulation of their behaviour.

van Emden and Harrington (2007) focused on the first two categories and identified studies that used natural enemies in the control of aphids as presented in the table below.

Table III: natural enemies used for the control of *Aphis gossypii*

Categories	Types	Species	Crop	Country
Augmentative release	Ladybirds	*Coccinella undecimpunctata*	Okra	Egypt
		Coccinella septempunctata and *Hippodamia variegate*	Cucumber	Morocco
		Hippodamia convergens	Straw berry	Belgium
		Harmonia axyridis	Cucumber	Japan
		Coccinella septempunctata and *Propylea japonica*	Cotton	China
	Predatory midge	*Aphidoletes aphidimyza*	Cucumber	UK
	Parasitoids	*Aphidius matricariae*	Cucumber	UK
		Aphidius colemani	Cucumber	UK
				Netherlands
				Italy
			Melon	France
		Ephedrus cerasicola	Cucumber	Norway
	Lacewings	*Chrysoperla carnea*	Okra	Egypt
			Cotton	Egypt
		Chrysoperla lucasina	Melon	France
Introductions or classical		*Aphidius matricariae*		France to Brazil and Chile
		Aphidius colemani		
		Aphidius matricariae		
		Aphidius picipes		
		Ephedrus plagiator		

Source: van Emden and Harrington (2007)

In Sudan, Abdelrahman *et al.* (1998) showed that natural enemies can contain aphid populations until an advanced stage of flowering of the crop. One of the main factors in the control of aphids is the entomopathogenic fungus *Neozygites fresenii* (Silvie and Papierok, 1991). Natural enemies play a significant role, but they cannot contain the aphid explosions in favourable climatic conditions.

I.4.3. Plant extracts and botanicals used against aphids

Aqueous Neem Kernel Extract (ANKE) and NeemAzal have been found to lower aphid numbers on okra (Mohamed-Ahmed, 2000). Neem Seed Kernel Extract (NSKE) (5%) is effective against *Aphis gossypii* on okra (Mishra and Mishra, 2002; Mudathir and Basedow, 2004). The effectiveness of botanicals was also confirmed by Adilakshmi *et al.* (2008) who worked on 8 botanical pesticides namely, Neemazal T/S (1%), Neemazal F (5%), NSKE, Vanguard (0.15%), Niconeem (0.03%), Neemol (0.03%), Neem oil and Gronim (0.15%). These evaluated botanicals were inferior to standard check (endosulfan), but proved superior to untreated check against sucking pests of okra. Mochiah *et al.* (2011) presented an array of botanicals that could significantly reduce pest populations, including aphids, and conveniently maintain ecological balance with their natural enemies on okra and eggplant. In their study, seven botanical treatments were applied *viz*, Ecogold (10 mL/l of water), Alata soap (5 g/l of water), Garlic (30 g/litre of water), Neem oil (3 mL/l of water), Papaya leaves (92 g/l of water), Wood ash (10 g/plant stand) and control (no botanical). For Okra, the percentage reduction in pests, including *A. gossypii*, because of botanical applications ranged between 42.8 to 76.9% depending on the substance used. In Africa and some continents, neem extracts containing azadirachtin (3 g active ingredient/ha) have been reported to be effective against aphids of okra (Ahmed, 2000; Praveen and Dhandapani, 2002; Obeng-Ofori and Sackey, 2003). Two doses of Azadirachtin tested by Nderitu *et al.* (2008) were found to offer okra protection from aphid though the efficacy was lower than imidacloprid.

I.4.4. Chemical control of aphids

It was known that the insecticides dominating aphid control were organophosphates (OPs), carbamates, and pyrethroids (Schepers, 1989; Jeschke *et al*., 2002). Although OPs and carbamate aphicides are systemic, relatively persistent and highly toxic to the target pests, they

are very toxic for beneficial insects, and many have since been withdrawn. In most cases, pyrethroid insecticides have replaced OPs, but their lack of systemic activity and broad-spectrum effects on non-target insects makes them even less suitable candidates than OPs as aphicides, even though their rapid action can sometimes prevent primary infection with some viruses.

Casida and Quistad (1998) reviewed the 'Golden Age' of these insecticides about two decades ago, Ishaaya and Horowitz (1998) then described compounds with new modes of action. This was stimulated by the demand for safer insecticides and the development of some new classes of chemicals. Some of the latter including the neonicotinoids, pymetrozine and triazamat had properties that were ideal for aphid's control. Carbamates, OPs, and pyrethroids were also important in many crops; this was reflected (especially with pyrethroids) in the choice available for the control of pests of crops. The neonicotinoids were approved for use in an increasing number of crops in many countries around the world, but have recently received band while pymetrozine and triazamate are still restricted especially in Europe.

Neonicotinoids were first discovered in the early 1970s, but they were not developed for use in agriculture until 1991, when imidacloprid (Elbert *et al*., 1990; Altmann and Elbert, 1992; Shiokawa *et al*., 1994) was introduced to the market as the first of the second-generation neonicotinoids (Jeschke *et al.,* 2002). Imidacloprid had the required photo stability, insecticidal activity, and residual persistence to be marketed for a wide range of uses and, in the past decade, has become the largest selling insecticide. It is a broad-spectrum insecticide; its excellent systemic action makes it ideal for controlling aphids. Other insecticides developed within this group include acetamiprid (Takahashi *et al.,* 1992), clothianidin (Ohkawara *et al.,* 2002; Jeschke *et al.,* 2003), dinotefuran (Wakita *et al.,* 2005), nitenpyram (Kashiwada, 1996), thiacloprid (Elbert *et al.,* 2000; Jeschke *et al.,* 2001), and thiamethoxam (Senn *et al.,* 1998; Hofer *et al.,* 2001; Maienfisch *et al.,* 2001). Pymetrozine was first reported in 1992 as CGA 215'944 (Flückiger *et al.,* 1992). It is a pyrimidine azomethine and thus has a new mode of action; it is highly active against aphids and whiteflies. Triazamate was first reported as RH 7988 in 1988 as a highly selective aphicide, with activity against many species (Murray *et al.,* 1988). Unfortunately, recently it was withdrawn from Europe. However, resistance by the cotton aphid to chlorinated hydrocarbon, organophosphate, and pyrethroid insecticides is widespread.

Several authors have documented evidence that insecticide resistance in cotton aphid is closely linked to elevation of carboxylesterase. Owusu and Yeboah (2007) worked on

carboxylesterase activity of *Aphis gossypii* populations from 20 locations in Ghana and found insecticide resistance in 18 locations. The use of insecticides should be sparingly and in conjunction with other non-chemical control methods to decrease the development of resistance. Various aphids' population susceptibility to endosulfan, esfenvalerate, methomyl, and oxydemeton-methyl in Hawaii were studied (Hollingsworth *et al*., 1994). The study found endosulfan as the best choice for aphid control; however, this product is among the pesticides restricted from use in Cameroon (MINADER, 2013). Anitha and Nandihalli (2009) reported higher efficacy of imidacloprid 70WS and 200 SL against aphids and leafhopper, in line with the findings of Day *et al.* (2005) who indicated that this chemical provided excellent protection against these organisms up to 45 days after sowing. Sreelatha and Divakar (1997), Krishna Kumar *et al.,* (2001), Nauen and Elbert (2003) also published similar results. This synthetic pesticide is also more effective than some useful botanicals (Nderitu *et al.,* 2008). Susceptibility to synthetic insecticides can be affected by difference in *A. gossypii* populations; for example, those from melon have been found to be more susceptible to imidacloprid than cotton aphids (Wang *et al.,* 2002; Hugh *et al.,* 2003; Tabacian *et al.,* 2011). In addition to leaf distortions by downward curling caused by aphid feeding, these mostly infest the lower surface of leaves that provide excellent shelter for the insects; in this case, systemic insecticides are useful. Young plants may have reduced or stunted growth that is damaging to the crop and may lead to total yield loss. When they are still very young, aphid infestations are often spotty, and if such plants or areas receive timely treatment, it will prevent great damage permitting them to develop some vigour that will be able to tolerate the pest even at high infestation levels. In vigorous varieties, a single application of synthetic chemicals can keep the crop tolerant for the rest of its growth. However, the use of insecticides for other more damaging insects sometimes leads to outbreaks of the cotton aphid (Hillocks (1995). Destruction of beneficial insects is purported to explain this phenomenon. Resistance to pesticide and destruction of beneficial organisms are major setback during chemical control including effects on environment and human health. The existence of obsolete ones necessitated the revision of pesticide registered for pest and disease control. The Europien Union (UTZ Certified, 2015) has banned clothianidin, thiamethoxam and imidacloprid (Neonicotinoids). MINADER prohibited the use of the following chemical: Captafol, Acetate De Dinosebe (Aretit), Dinosebe, Binapacryl (Morocide), Cyhexatin, Dieldrine, Aldrine, Heptachlore and 2-4-5 TCP in Cameroonian markets in 1998, Lindane in 2005, Malathion, Amitraz, Carbaryl,

Cartap, Diazinon, Endosulfan, Fenobucarb (BPMC), Methyl-parathion, Propoxur on cocoa in 2008, Carbosulfan in 2009 and Diméthoate in 2011 (MINADER, 2013).

I.4.5. Okra resistance to aphids

The use of host plant resistance is a core component of integrated pest management. This is because of the fact that chemical pesticides will be reduced on tolerant and moderately resistant varieties. This method also allows the proliferation of potential natural enemies, and other habitat management options such as intercropping and crop rotation could be incorporated. The availability of aphid-resistant okra genotypes has been confirmed (Uthamasamy *et al.,* 1976; Gunathilagaraj *et al.,* 1977; Sumathi, 2005; Anitha and Nandihalli, 2009), although they are limited (Dogimont *et al.,* 2010). Apart from okra, resistance to aphids has also been reported in leguminous crops (Hill *et al.,* 2004; Mensah *et al.,* 2005) and cereals (McCreight, 2008; Collins *et al.,* 2005). Recently, Dogimont *et al.* (2008) identified the *Vat* allele, which is responsible for melon resistance to *Aphis gossypii;* this author also showed that crop resistance to this insect was biotype specific. Some earlier studies had identified biotypes of *A. gossypii* (Guldemond *et al.,* 1994); although those of the melon/cotton aphids are morphologically indistinguishable, they have distinct host ranges. Efforts have shown some differences in host preference (Wang *et al.,* 2004), feeding behaviour (Gutierrez *et al.,* 2008) and virus transmission (Yokomi *et al.,* 2004) between the melon and cotton biotypes. *Aphis gossypii* from cotton has a lower reproduction on cucurbits, eggplant (*Solanum melongena),* sweet melon (*Cucumis melo),* okra and *Sesamum indicum* (Moursi *et al.,* 1985).

Little knowledge has been provided for the mechanisms and categories of resistance. Leaf nitrogen level has been reported as an indicator of food quality and a factor of host selection by herbivores (Mattson, 1980). Metabolites such as carbohydrates and proteins and free amino acids are also important for the development of *A. gossypii* (Slosser *et al.,* 1989; Deguine and Hau, 2001). Plants produce a high diversity of natural compounds or secondary metabolites with toxic nature and repellence to herbivores and microbes. Some of the chemicals are also involved in defence against abiotic stress and are important for the communication of the plants with other organisms (Schafer and Wink, 2009). Lu *et al*. (2009) found that tannin content was negatively correlated with *A. gossypii* resistance in cotton. Zucker (1982) found an inverse correlation for the effect of total phenols in the tree, *Populus anqustifolia*, to a galling aphid, *Pemphigus betae*.

Jenkins (1989) and Watson (1989) had reported that the degree of trichome density on the leaves of cotton is related to degrees of resistance/susceptibility to aphids and jassids. Lee (1985) had stated that the primary source of resistance in cotton to sucking insect pest is the presence of trichomes. It was also found that the leaf trichome density in okra affects *A. gossypii* (Deguine and Hau, 2001; Leite *et al.*, 2007). Scriber and Feeny (1979) showed that leaf toughness was also involved in making the leaves progressively less suitable as they age.

I.4.6. Integrated Pest Management (IPM) of aphids

According to van Emden (2007), the foundation of Integrated Pest Management (IPM) in the present era of pest management was laid by the concept of Stem *et al.* (1959) called Integrated Control (IC). This concept was formulated to integrate chemical and biological methods for the control of *Therioaphis trifolii maculata* (spotted alfalfa aphid) on Lucerne (alfalfa), *Medicago sativa*; this means IPM began with aphids. Due to a rapid appearance of resistance of spotted alfalfa aphid in the late 1950s to OPs compounds, that also killed the indigenous natural enemies, a reduced dose of an OP insecticide was integrated in Califonia, with the biological control. Pest Management (PM), another common term used in plant protection was created as a successor to IC during a conference at Raleigh, North Carolina, USA, in 1970 (Beirne, 1970). The use of both single and multiple control measures was considered PM but IPM emerged later in the 1970s (Apple and Smith, 1976) and described to include all categories of pests such as pathogens, insects, nematodes, and weeds. This confusion in attributing the “I” in IPM to integration of plant protection discipline such as entomology, phytopathology, nematology and malherbology was clarified from 1976 onward, and nowadays IPM seems indistinguishable from PM, or even from IC. The main components of IPM systems for aphids are developed based on sources of mismanagement such as overdosing resulting in tolerant pest populations, loss of beneficial organisms, and use of high-yielding but pest-susceptible monoculture crops and lack of labour-intensive cultural controls. This implies that chemical control should be applied in a way that keeps the pests below economic thresholds. This way, biological control is conserved especially when selective pesticides are used. Other components are the use of partially aphid-resistant crop varieties and introduction or re-introduction of cultural controls in order to improve conditions for indigenous natural enemies (Wratten *et al.*, 2007). Recent contribution has come from techniques such as the use of semio-chemicals to

modify the behaviour of aphids and their natural enemies in IPM strategies. van Emden (2002) explained the importance of IPM using it's golden rule by pointing out the danger that could arise if a single method gives adequate control on its own; that tolerant pest strain could arise and no opportunity to use a second method in addition may exist. The efficiency of the method therefore needs to be lessened, for example through a reduced dose of pesticide, partial host-plant resistance rather than immunity, for there to be need in introducing another control method to supplement it. A second rule is that methods are worth combining to the extent that the control achieved exceeds the additive effects of the two methods in isolation.

A true integrated approach is recommended for aphid management, with a number of cultural practices likely to limit the incidence of end-of-cycle infestations, including early sowing, rational fertilization, early picking (Deguine, 1995), and spraying to economic thresholds. In order to implement these IPM techniques, scouting, sampling, and trapping populations of *Aphis gossypii* are required. In Cameroon, an attractant panel of techniques for trapping winged forms of *A. gossypii* has been developed (Deguine and Leclant, 1996). The system is effective, reliable, inexpensive, and simple to establish in the field. This permits monitoring of alatae activity at the beginning of the farming season, making it possible to incorporate appropriate control measures in IPM programmes (van Emden and Harrington, 2007).

CHAPTER II: MATERIALS AND METHODS

II.1. Study site

II.1.1. Genetic studies of aphid populations

Genetic studies were conducted in the Biotechnology laboratory of AVRDC in Taiwan (Figure 16).

II.1.2. Screening and identification of aphid-resistant okra germplasms

Four preliminary screening trials were conducted at AVRDC campus in Shanhua, Taiwan (23°08.29'N, 120°19.15'E). Six trials were conducted at AVRDC Cameroon based at IITA campus in Nkolbisson, Yaoundé, Cameroon (03°51.79'N, 11°27.71'E) (Figure 16). The six trials were one preliminary screening, three advanced replicated screenings and three confirmatory screenings.

Figure 16: AVRDC offices in the world (http://www.avrdc.org/index.php?id=8).

II.1.3. Biophysical and biochemical studies

Biophysical bases of resistance for the first selection (year 2012), second and third selection both in 2013 were conducted in Taiwan, while combined studies involving all selections were realised in Cameroon in 2014. All studies on biochemical bases of resistance were conducted in the entomology and nutrition laboratories of AVRDC in Taiwan.

II.1.4. Mechanisms of resistance

For the mechanisms of resistance, studies on tolerance were conducted in Cameroon. Those for antixenosis were conducted at AVRDC campus in Taiwan for varieties selected in the first replicated trial (2012) and the second and third replicated experiments (2013). Studies involving the combination of all the three experiments were carried out in Cameroon, while those on antibiosis were all conducted in screenhouse at AVRDC Cameroon.

II.1.5. Aphid resistance and yield performance of okra accessions under various agro-ecological climates in Cameroon

II.1.5.1. Geographic positioning system data

Multilocation trials were conducted in four of the Cameroon's five major agro-ecological zones (Figure 17) that cover the 10 administrative regions of the country.

The following four experimental sites were choosen based on preliminary survey of aphids on okra conducted in 2011 in Camerroon. From the survey results, these locations were idenfied as major okra production sites and hot spots for aphid infestion within their respective ecological zones:

- Buea has a tropical rainforest with a tropical monsoon climate at the foot of Mountain forest and fall within zone IV (Warm and humid forest with monomodal rainfall). However, the southern part of this zone has four seasons up to acroos the river Nyong. It is located at 04º11.253′N, 009º18.849′E, and 480 m above sea level (a.s.l.);
- Evodoula has a humid and warm equatorial climate characterized by semideciduous evergreen humid forest, and falls within zone V (Warm and humid forest with bimodal rainfall relatively limited): 04º06.679′N, 011º11.228′E, 572 m a.s.l;
- Foumbot has a Sudano-Guinean tropical climate located on a vast plain with volcanic soils and situated within zone III (Cold and humid western savannah highlands with monomodal rainfall): 05º28.903′N, 010º35.539′E, and 1022 m a.s.l;
- Maroua climate is tropical, dry and warm, almost semi-desert and found in Zone I (Sudano-sahelian zone): 010º53.800′N, 014º24.572′E and 459 m a.s.l;

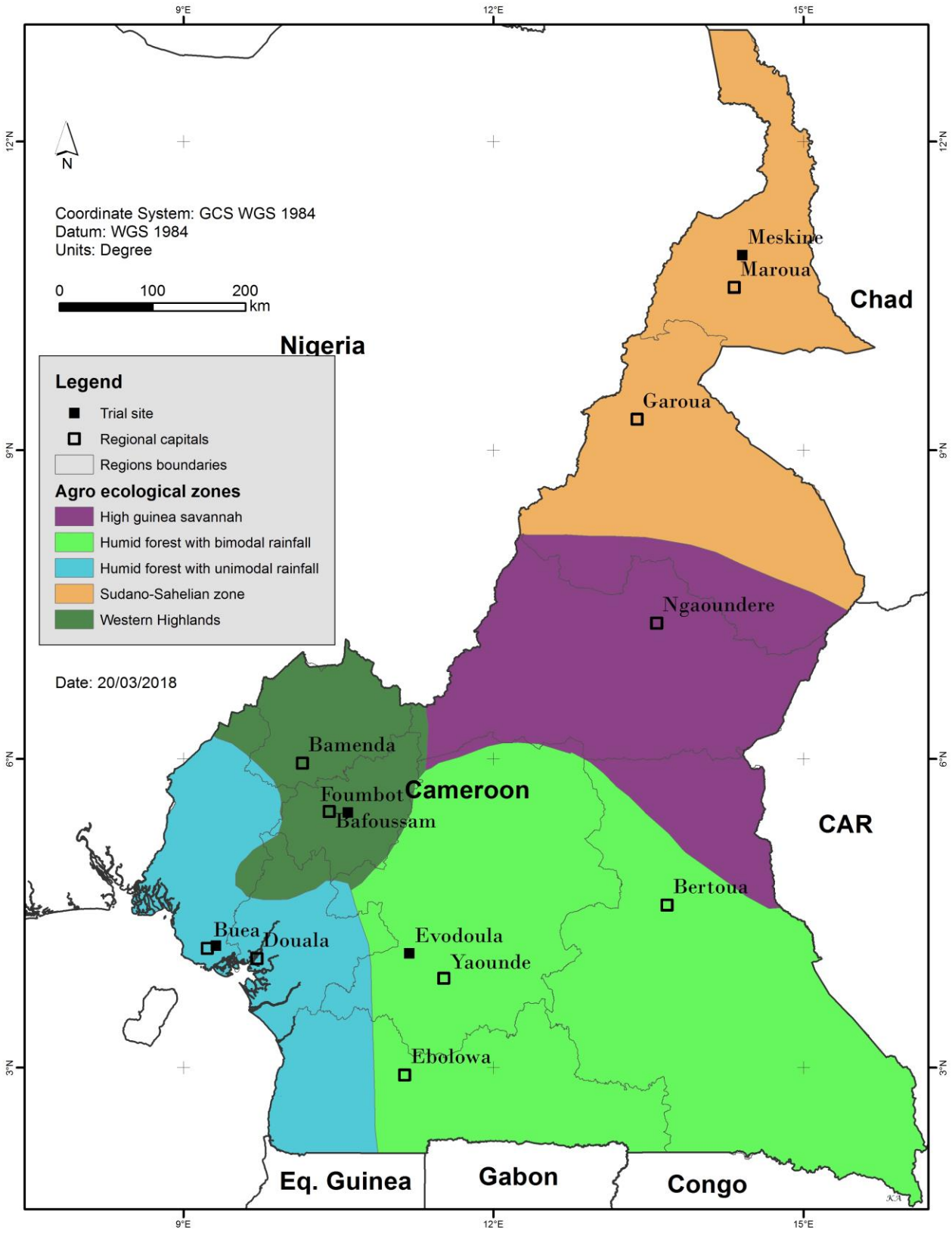


Figure 17: Cameroon map showing agroecological zones (Modified from IRAD, 2008).

II.1.5.2. Climate of the four sites during the study periods

Buea is a tropical rainforest with a tropical monsoon climate at the foot of Mountain forest. As expected, the rainfall pattern in Buea was monomodal reminicent of its monomodal humid rain forest on Cameroon's ecological zone IV. However, the southern part of this zone has four seasons up to the mouth of river Nyong.

During the trial, temperatures ranged from 21.7 to 26.2°C, rainfail from 65 to 488 mm and RH from 83.6 to 94.7%. Peak rainfall was between July and September and lowest between October and April. All periods of the trials were wet (P > 3T) (P: Precipitation, T: temperature) except December that was semi wet period (3T > P > 2T) (Figure 18).

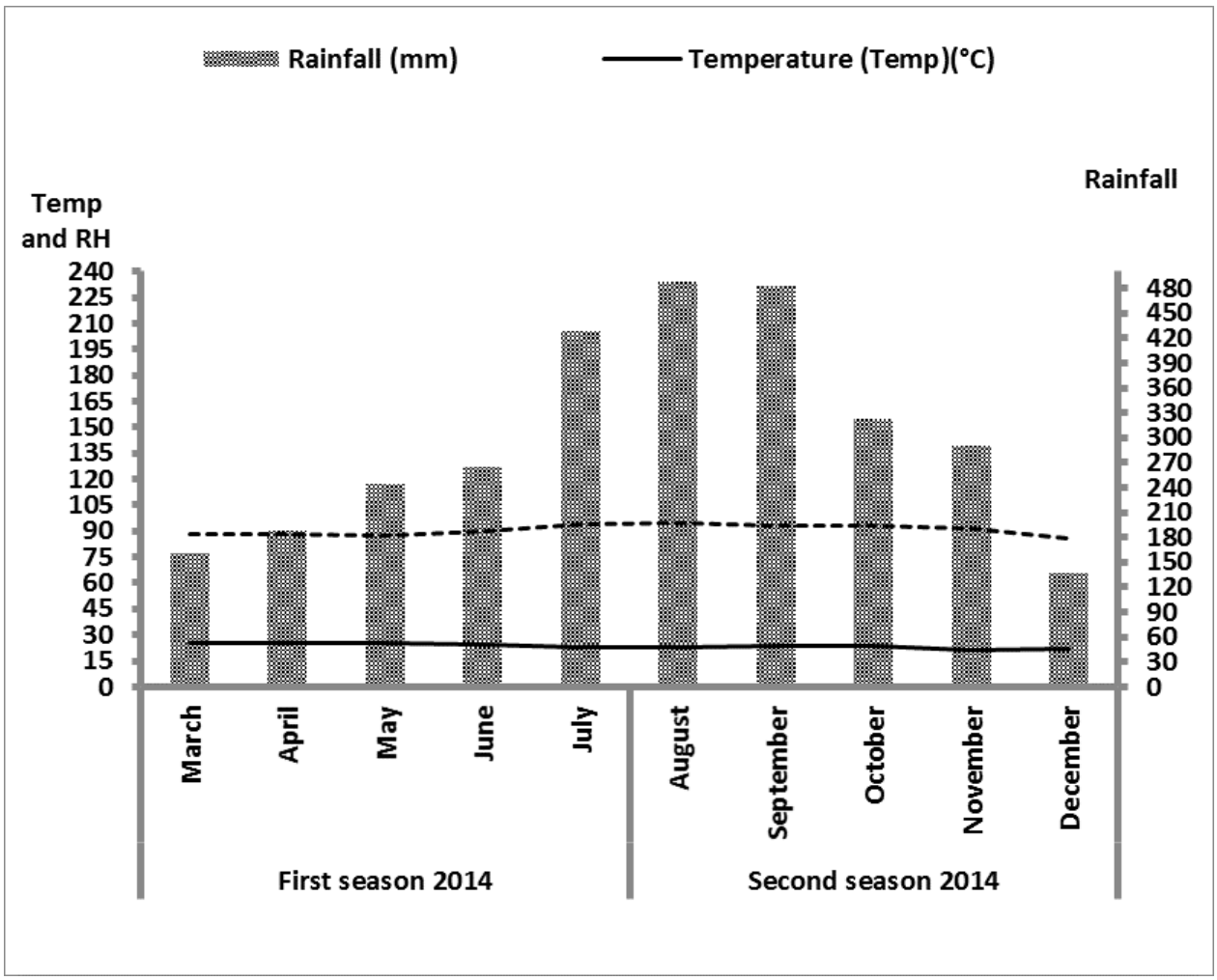


Figure 18: parttern of climatic factors for the monomodal warm and humid forest of Buea (source: IITA Cameroon).

Evodoula has a sub equatorial climate with relatively low rainfall an element of semi-deciduous forest in contact with the southern evergreen forest. This site is found in the Center region with a bimodal humid rain forest of Cameroon's zone V. Similarly, the rainfall pattern showed two peaks during the year of study. It was as expected and there were two dry periods during the year (P < 2T). During the trial, temperatures ranged from 22.2 to 24.7°C, rainfail from 28.7 to 385.1 mm and RH from 84.7 to 94.0% (Figure 19).

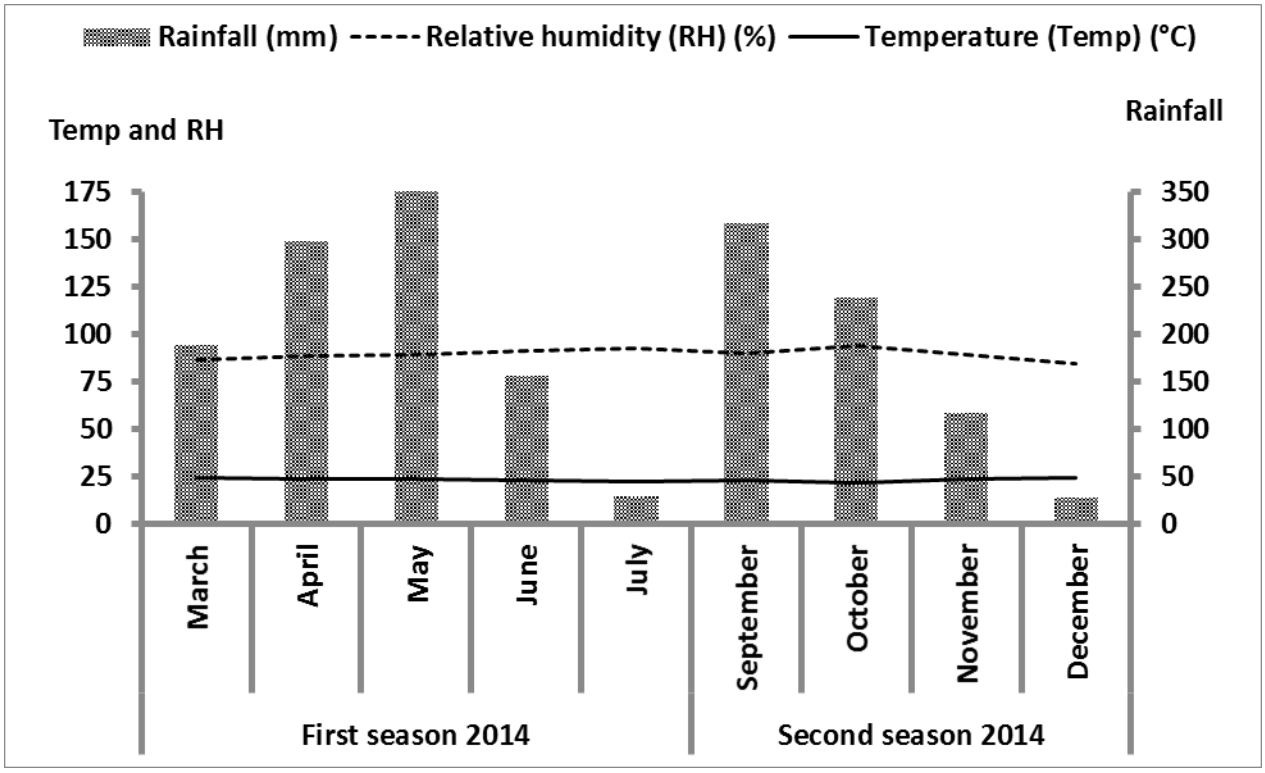


Figure 19: parttern of climatic factors for the bimodal warm and humid forest of Evodoula (source: IITA Cameroon).

Foumbot has a Sudano-Guinean tropical climate located on a vast plain with volcanic soils and situated within zone III. Like most of the western highlands, Foumbot shows a monomodal rainfall parttern with a dry period once a year from December (P < 2T) until March which was semi wet period (3T > P > 2T) (Figure 20). During the study period, temperatures in Foumbot ranged from 20.0 to 24.3°C, rainfall from 128.3 to 312 mm and RH from 84.2 to 88.6%. In Foumbot, the single rainy season showed a peak in October 2014.

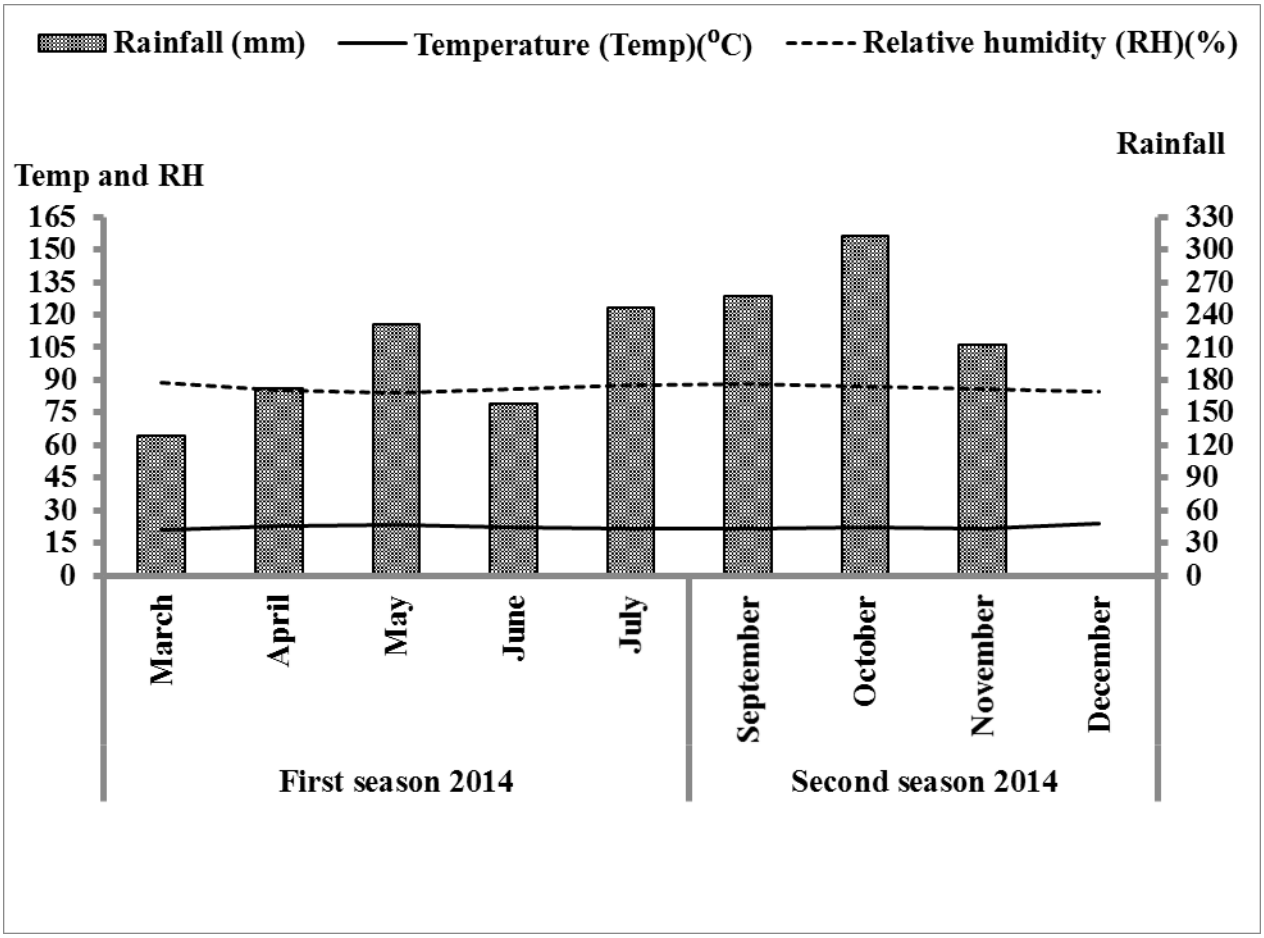


Figure 20: parttern of climatic factors for the monomodal western highlands of Foumbot (source: IITA Cameroon).

Maroua climate is tropical, dry and warm, almost semi-desert and found in Zone. The climatic parttern during the study showed a soudano-saelian pattern with one rainy season from May to October in 2013 and March to October in 2014. Temperatures ranged from 17.1 to 33.4°C, rainfall from 0 to 276.5 mm and RH from 23.4 to 93.6%. The wet periods was only June to September (P > 3T or 3T > P > 2T). All other periods of the year were arid (P < 2T) (Figure 21).

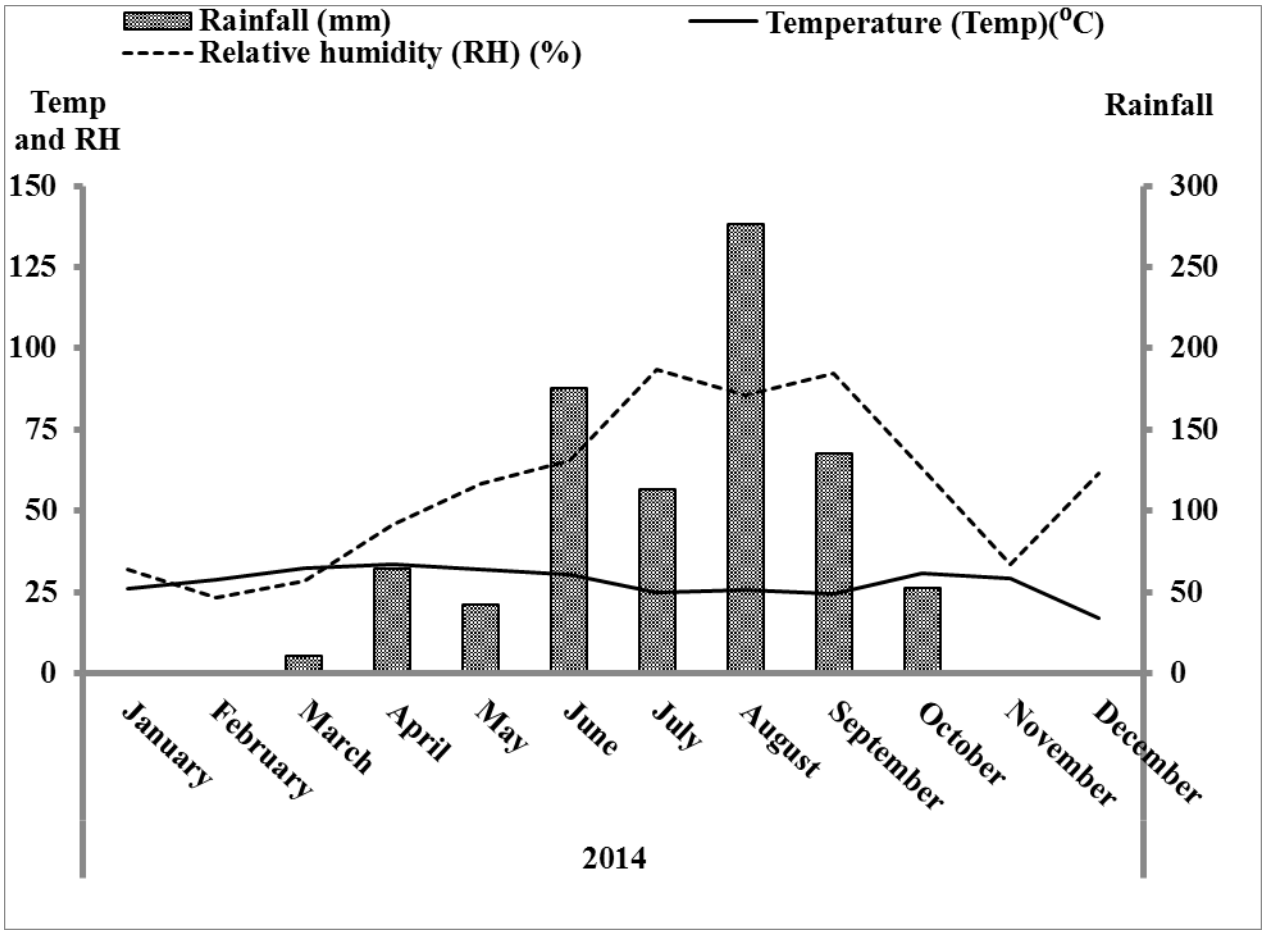


Figure 21: parttern of climatic factors for the soudano-sahelian zone of Meskine in Maroua (source: IITA Cameroon).

II.2. Biological materials

The 430 okra germplasms used during the preliminary screening experiments out of 445 (total number) were obtained from the Genetic Resources and Seed Unit (GRSU), AVRDC – The World Vegetable Center, Taiwan. The remaining 15 comprise 4 commercially available varieties in Cameroon purchased from seed stores and 11 varieties collected during a survey from different farmers' fields in Cameroon.

Aphids used for antixenotic analysis were harvested from neighbour okra field at AVRDC Cameroon for the Cameroon trial and from AVRDC Taiwan campus for Taiwan trials. Studies on antibiosis were all conducted in Cameroon, and so all sources of aphid colonies were from okra fields at AVRDC Cameroon campus.

For the multilocation experiments, the most common local variety identified by farmers from each location was used as checks. These were Gombo paysan in Buea, Bangourain in Foumbot, Kirikou in Evodoula, and Bosco Djo in Maroua. Gombo caféier was used as a common commercial variety in all location.

II.3. Methodology

II.3.1. Genetic study of *Aphis gossypii*

II.3.1.1. DNA Extraction

Six aphid populations from Taiwan and Cameroon, where preliminary screenings were done, were collected and stored in absolute or 90% alcohol. The populations were:

- aphids from okra field in the high altitude savannah of Mbengwi in North-West region, Cameroon (6°01.00’ N, 010°00.00’ E and 1260m above sea level);
- aphids from farmers’ okra field in warm and humid forest of Nkolbisson in Yaounde, Cameroon (03°51.990' N, 011°27.688' E and 765m above sea level);
- aphids from *Hibiscus* plant in warm and humid forest of Nkolbisson in Yaounde, Cameroon (03°52.081' N, 011°27.743' E and 753m above sea level);
- aphids from on-station okra in warm and humid forest of Nkolbisson in Yaounde, Cameroon (03°51.791' N, 011°27.706' E and 747m above sea level);
- aphids from on-station okra field at AVRDC campus at Shanhua in Taiwan (23°08’29”N, 120°19’15”E);
- aphids from okra field in India (11°59’N, 78°01’E);

Twenty individuals were put in a micro-centrifuge tube (1.5 mL) and 50µl of “Universal All™ Extraction buffer” were added. The tissue was ground using a pipette tip, centrifuged at 4000 rpm for 3 seconds (Figure 22a), and the mixture was incubated at 95°C for 10 min (Figure 22b). The solution was vortexed for 1 sec and centrifuged at 4000rpm for 2 to 3 sec. Three different concentrations of each population of the aphid DNA solution were prepared by transferring 10 µl each of the supernatant into 1.5 mL micro-centrifuge tube. The solutions were diluted with distilled water to 1:10, 1:20 and 1:40 ratios for each population, and reserved for the polymerase chain reaction (PCR).

(a) (b)

Figure 22: equipment used during DNA extraction; a: micro-centrifuge and b: incubator (picture by Abang, 2012).

II.3.1.2. Polymerase chain reaction

These aphid populations were compared based on the cytochrome c oxidase I (*cox I*) gene at AVRDC Taiwan. Four primer pairs were designed for the gene and tested on each population using the lower concentration of 1:40. The concentration was selected based on previous PCR conducted with the different concentration using the elongation factor primers 'EF3' (5'-GAACGTGAACGTGGTATCAC-3') and 'EF2' (5'-ATGTGAGCAGTGTGGCAATCCAA-3') or 'EF6' (5'-TGACCAGGGTGGTTCAATAC-3') (von Dohlen and Teulon, 2003). Four annealing temperatures (63.9, 61.0, 56.4, and 52.5°C) were tested and lower temperatures produced better bands. However, *cox I* was used for the PCR product that was used for sequencing of the six aphid populations DNA, because it is genetically stable due to its location in the mitochondria. The annealing temperature of 50°C was choosen based on the previous PCR using the Elongation factors. The four primer pairs were:

Ago_CoxI_f1 (5'-CAATCGTTATTGGAGGTTTTGG-3'),

Ago_CoxI_r1.1 (5'-ATGTGAAGTAGGCTCGTGTATCTA-3'),

Ago_CoxI_f2 (5'-CTTACCTGTATTAGCTGGTGCTAT-3'),

Ago_CoxI_r2.1 (5'-GTTCTAATGGTGGAAGATTGTG-3'),

Ago_CoxI_r2.2 (5-TTCGGGTAATCTGTATATCGTC-3') and

Ago_CoxI_r1.2 (5'-TATAGTTGCTGATGTGAAGTAGGC-3').

Corresponding volumes in micro-liters of TE buffer were added to dissolve each primer and obtain a concentration in 100 µmole. The bench concentration was obtained by making a 1:10 dilution of 10 µl of the dissolved primers with 2D water. Components of the primary cocktail were 5.7 µl water, 1.5 µl 10X Super-Therm Gold Buffer, 1.2 µl 2.5 mMdNTP mixture,

0.6 µl 25 mM $MgCl_2$, 1.5 µl 10 mg/mL BSA, 0.3 µl 2 U/µl Super-Therm Gold DNA. However, a master mix of the components was made in which the volume of each component was multiplied by 24 (six populations × four primer pairs) increased by 10% (Figure 23a). Four secondary cocktails were made from the four pairs of primers by mixing 0.6 µl of each component of a primer pair. The volume of each of the secondary cocktails was multiplied six times (aphid populations). Twenty-four 0.5 mL micro-centrifuge tubes were prepared for the samples. To each tube, 3 µl of each aphid population DNA template was placed, 10.8 µl of primary cocktail and 1.2 µl of secondary cocktail added. The sample volume for PCR was 15 µl and the PCR was run for 35 cycles for 2 h 30 min in a thermal cycler (Figure 23b). Because sequencing would be done with the same PCR product, the final cocktail components were doubled and the PCR sample solution was 30 µl instead of 15 µl.

(a) (b)

Figure 23: Preparation and running a PCR: a = Studying procedure to conduct a PCR; b = thermal cycler used for PCR (picture by Abang, 2012).

II.3.1.3. Gel electrophoresis

Gel electrophoresis was conducted using 10 µl of the PCR product to which 2 µl of loading buffer were added in microfuge tubes. The remaining 20 µl of PCR product were reserved for sequencing. A 28 well gel was made with 1.6 g agarose dissolved in 80 mL TE (Tris EDTA) buffer to make a 2% agarose gel. The mixture was heated in a microwave for 1 min and stirred with rod on stirring plug for 5 min until the solution was transparent. The transparent solution was poured into a mould sealed on the sides. A comb with enough teeth that could take

the number of samples plus at least two marker lanes was inserted. After 30 min, when the gel had been formed, the comb and the seals were removed. The gel was placed into the gel box containing TE buffer, with the well facing the negative electrode of the gel box (Figure 24a). The PCR product, to which 2 µl of loading dye were added, was loaded into the wells using a pipette (Figure 24b), including one DNA marker lane each at the outer wells. Electrophoresis was run at 100 volts for 1 h. The gel was stained with ethidium bromide for 15 min and destained with distilled water for 7 min.

(a) (b)

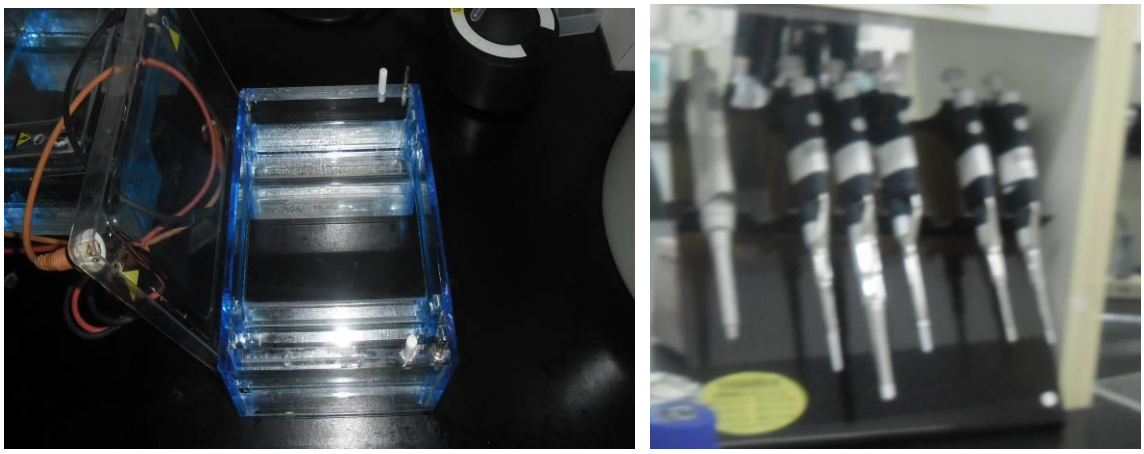


Figure 24: loading samples for electrophoresis; a = gel apparatus; b = pipettes (picture by Abang, 2012).

II.3.1.4. DNA sequencing and phylogenetic analysis

The PCR product was sent to Genomics BioSci Tech Co., Taiwan for sequencing with 20 µl of each sample in micro centrifuge tubes. Because two PCR product sizes were obtained, two primer pairs were also selected and used for sequencing. These were Ago_CoxI_f1and Ago_CoxI_r1.2 with relatively longer chain, and Ago_CoxI_f2 and Ago_CoxI_r2.1 whose PCR product size was about 900 bp. The retrieved sequence data including available *Aphis craccivora* nucleotide sequence at NCBI GenBank were aligned by the ClustalX2 program (Larkin *et al.*, 2007) and subjected for subsequent phylogram construction using MEGA5 and using the Kimura two parameter distance model (Tamura *et al.*, 2011).

II.3.2. Screening and identification of accessions resistant to *Aphis gossypii*

II.3.2.1. Preliminary screening trials

Preliminary screening was conducted in non-replicated trials with ten plants per ridge. The seeds of each variety were sown three per hole (1 m apart) in a single row on ridges of 10m x 1m with 0.5 m between ridges and thinned to single stands at 2 weeks after sowing. Five preliminary screening trials were conducted during the current study. Three in 2011, *viz*., March to May (88 accessions), September to November (68 accessions) and November 2011 to February 2012 (112 accessions); two in 2012, *viz*., March to May (96 out of 107 accessions since 11 did not germinate), September to November (64 accessions). The trials in each country were carried out in one field in each season. They were maintained following customary cultural practices and without pesticide application, to control aphids or other sucking insects. Hibiscus plants were planted around the screening plot to increase pest pressure and the first ridges all round the screening plot were exempted from sampling to minimize boarder effects. The trials were exposed to the natural infestation of aphids and the pest populations were directly scored at weekly intervals starting from four weeks after sowing in the field.. Five plants of each accession were randomly selected from the middle of the ridges. On each plant, three leaves were randomly selected, one each from bottom, middle and top strata of each plant to record the number of aphids and other insects present. Aphids were scored using the following rating scale: 0 = no aphids present; 1 = 1 to 10 aphids per leaf; 2 = 11 to 100 aphids per leaf; 3 = 101 to 500 aphids per leaf; and 4 = > 500 aphids per leaf (AVRDC, 1979).

II.3.2.2. Advanced replication screening trials

The selected resistant okra accessions from the first three preliminary screening trials conducted in Cameroon and Taiwan in 2011, with the known susceptible control (VI057245) (Appendix 1), were screened in first advanced replicated screening trial in Cameroon during March–June 2012. Four accessions rated aphid-resistant from the fourth preliminary screening in spring 2012 in Taiwan, including the 11 varieties collected from different farmers' fields in Cameroon, were evaluated in the second advanced replicated trial from October 2012 to March 2013 in Cameroon. Seven accessions rated aphid-resistant from fifth preliminary screening trial were evaluated in the third advanced replicated trial from March to July 2013 in Cameroon. All three advanced replicated trials were conducted in a randomized block design (RCBD) with three

replications. The crop management and screening methods were similar to the preliminary screening trials.

II.3.2.3. Confirmatory screening trials

Accession that were rated as resistant or moderately resistant to aphids from all advanced replicated trials, were screened in confirmatory screening in two seasons (March to July 2014 and September to December 2014) together with the known susceptible accession (VI057245) (Figure 25). VI060794 was also considered as a second susceptible check following its high susceptibility in the final confirmatory trial in Taiwan in 2013. The farmer popular local variety Kirikou dominating the market in Mfoundi division and Gombo caféier a Cameroonian common commercial variety were considered as farmers' checks. The advanced confirmatory trials were conducted using a randomized block design (RCBD) with three replications. The management and screening methods were similar to the advanced replicated screening trials.

Figure 25: on-station screening field at Nkolbisson, Yaounde, Cameroon (picture by Abang, 2014).

II.3.3. Biophysical and biochemical characteristics of okra resistance

The plants for studies of biophysical and biochemical characteristics of okra were sown in plastic trays and single plants transplanted at first true leaf stage (2 weeks after sowing) (2WAS) in clay pots (Height 25 cm and diameter 20 cm) containing 50% soil + 25% Sand + 25% fowl

manure (Figure 26a). The potted plants of each accession were arranged in completely randomized design (CRD) in screen houses, in 5 replications of 2 plants each (Figure 26b).

For biophysical studies, three leaves, one each from the top (leaf 1), middle (leaf 2) and bottom part (leaf 3) of the plant were collected for this study at the beginning of flower bud initiation. For biochemical studies, all six leaves from plants, two each from the top (leaf 1 and 2), middle (leaf 3 and 4) and bottom (leaf 5 and 6) of the plant were collected at 6 WAS and at 10 WAS and dried at -56°C for seven days in a freeze dryer. The dry samples were ground in an electric blender. The samples were replicated five times for each accession, with two plants per replication. To investigate whether the plant metabolites involved in resistance or susceptibility to pests are constitutive or change following attack, as induced response (Khattab, 2007; Wilson *et al.*, 2011), other potted plants were prepared in a similar manner but were previously infested with 10 aphids each at vegetative stage, and 25 aphids at reproductive stage. Five days later, the aphids were removed using a soapy solution prepared by mixing one teaspoon of liquid savon (TRI Shine Lime Dish Liquid) in 1 L water, and sprayed on okra leaves. The leaves were rinsed with water used 24 h later to collect samples for biochemical analysis (Messina and Bloxham, 2004).

(a) (b)

Figure 26: okra plants; a= nursery with plants at first true leaf stage; b = potted plants (picture by Abang, 2012).

II.3.3.1. Biochemical studies

- Tannins

Analysis of okra leaf tannins was carried out using the catechin standard and acidified vanillin method (Broadhurst and Jones, 1978). One gram of sample powder mixed with 10 mL

(V_1) of 99.9% methanol in screw cap bottles, and vibrated for 1 h in an orbital shaker (Figure 27a). The weights of pairs of sample mixtures were balanced using a scale and centrifuged for 10 min, at 10000 rotations per min (rpm) in a macro-centrifuge (Figure 27b).

(a) (b)

Figure 27: extraction of tannins; a: orbital shaker; b: macro-centrifuge (picture by Abang, 2012).

The supernatant was collected and precipitate was discarded. One mL of sample was diluted with 2 mL of 99.9% methanol to make 3 mL (V_2). Catechin concentration of 0.5 mg/mL was used as standard by dissolving 25 mg of catechin in 50 mL of methanol, depending on the amount of water molecules present. Separate volumes of 0, 0.2, 0.4, 0.6, 0.8 and 1 mL of standard were taken and made up to 1 mL by adding methanol. The reagents used were 1% Vanillin, 4% and 8% Hydrochloric acid (HCl) prepared by adding Methanol based on the concentration of HCl. To one test tube, 5 mL (V_3) of 4% HCl were added to 1 mL (V_4) methanol and set as reagent Blank. The 1% vanillin and 8% HCl were mixed at the ratio 1:1, and 5 mL (V_3) of the mixture were added to 1 mL (V_4) of each diluted plant samples and the different standard concentrations in separate test tubes. All test tubes containing the reagent blank, the standards and the sample mixtures were vortexed at 100 rpm and incubated at 30°C in water bath for 20 min. The absorbance was determined using a spectrophotometer at 500 nm. The concentration of the standards was adjusted if need be to get an optical density (O.D) value of the sample concentration of tannins closer to the average. The R^2 value most be approximately 1 as shown in the linear regression of a standard curve (Figure 28) by ploting absorbance against concentration

as follows: Y = AX + B, where A = gradient of standard curve, X = Net sample OD, B = Intercept. R^2 = 0.9993 (square root of Pearson's coefficient of correlation or proportion of the variance in y attribute to the variance in x is approximately 1). The tannins content was determined using the formular Y=0.6789X-0.002, Tannin content = Y × V_1/V_3 × V_2/V_4 in mg/g (Broadhurst and Jones, 1978).

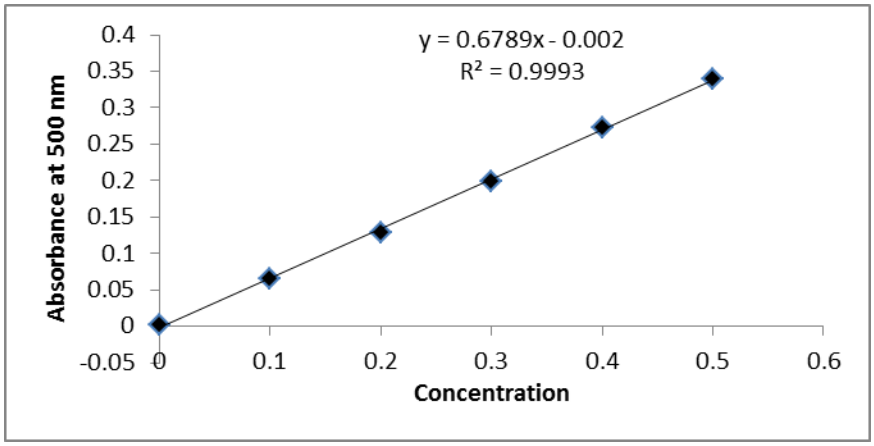


Figure 28: standard curve for concentration of tannins standard solutions against OD values.

❖ Total sugars

Total sugar content of okra leaves was spectrophotometrically determined using the Anthrone reagent (Sigma, A1631), in a method described by Dreywood (1946). Anthrone reagent reacts specifically with carbohydrates in the concentrated sulphuric acid solution to produce a blue green colour at 630 nm. The results were expressed as sucrose equivalents. D (+)-Glucose anhydrous was used as standard by dissolving 50 mg in 500 mL distilled water. Glucose standard of 0, 8, 16, 24, 32, 40 and 48 (ppm) were prepared by measuring 0, 0.2, 0.4, 0.6, 0.8, 1 and 1.2 mL glucose stock in separate test tubes and volume made up to 2.5 mL (V_2) with distilled water. Using a 35-mL centrifuge tube, 100 mg (Ws) of sample powder was heated with 10 mL of 80% ethanol in a water bath (Figure 29a) at 80 to 85°C for 30 min. The sample was then cooled to room temperature and centrifuged for 10 min at 10,000 rpm. The supernatant was collected and the extraction step was repeated twice with the residue and all the supernatant was collected into tubes. The supernatants were concentrated to 3 mL at 80 to 85°C in a water bath. The concentrate was diluted to 250 mL (V_1) and 2.5 mL (V_2) were taken from both the dilution and the standards into respective tubes, placed in ice bath and 5 mL 0.2% Anthrone was slowly added and mixed by vortex.

(a) (b)

Figure 29: water baths: a: for extraction and b: for incubation (picture by Abang, 2012).

The samples were transferred into boiling water bath and incubated (Figure 29b) for 7.5 min and cooled to room temperature. The absorbance was read using a spectrophotometer (U-2001, Hitachi, apan) at OD 630 nm. Concentrations of standard solutions were adjusted if necessary to obtain OD values of sample solutions closer to average value of the standards and with an R^2 value approximately 1 as shown in the linear regression curve (Figure 30). Total sugars were determined using the standard curve Y = AX+B, where A = gradient of curve, X = OD, B = intercept, R^2 = 0.9943 (coefficient of correlation is approximately 1)

Percentage carbohydrates = $Y/1000000 \times (V_1/V_2)/Ws \times 100\%$ (Dreywood, 1946).

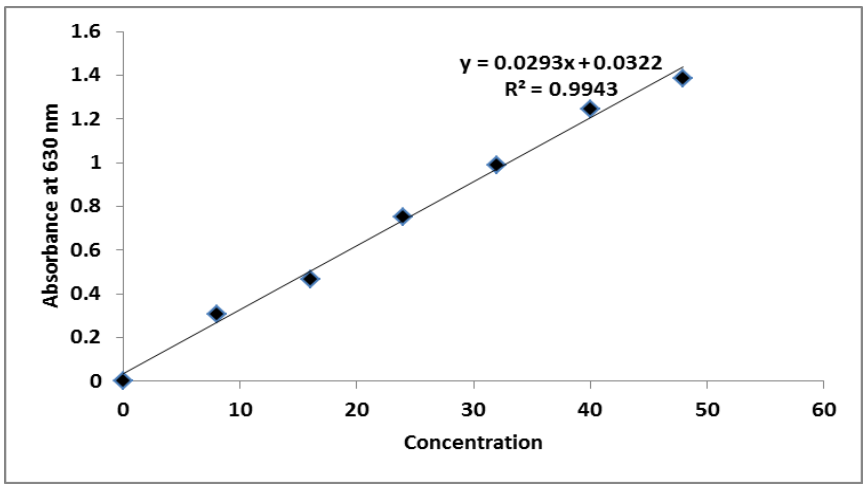


Figure 30: standard curve for concentration of total sugars against standard solutions OD values.

❖ Reducing sugars

Determination of reducing sugars was done by Nelson-Somogyi Method (Somogyi, 1952). They are sugars with reducing property (arising out of the presence of a potential aldehyde or ketone group). Some of them are glucose, galactose, lactose and maltose. The Nelson-Somogyi method is one of the classical and widely used methods for their quantitative determination. The reducing sugars when heated with alkaline copper tartrate reduce the copper from the cupric to cuprous state and thus cuprous oxide is formed. When cuprous oxide is treated with arsenomolybdic acid, the reduction of molybdic acid to molybdenum blue takes place. The blue colour developed was compared with a set of standards in a colorimeter at 620 nm. To prepare the Alkaline Copper Tartrate, 2.5 g of anhydrous sodium carbonate, 2 g of sodium bicarbonate, 2.5 g of potassium sodium tartrate and 20 g of anhydrous sodium sulphate were dissolved in 80 mL water and made up to 100 mL to form solution A. Then 15 g of copper sulphate was also dissolved in a small volume of distilled water, and one drop of sulphuric acid was added and the volume was made up to 100 mL to form solution B. Four litres of B and 96 mL of solution A were mixed before use. To prepare arsenomolybdate reagent, 2.5 g of ammonium molybdate were dissolved in 45 mL water. 2.5 mL of sulphuric acid were added and mixed well. To this solution, 0.3 g disodium hydrogen arsenate dissolved in 25 mL water was added. The solution was mixed well and incubated at 37°C for 24h. The standard glucose stock solution was prepared by dissolving 100 mg of glucose in 100 mL distilled water. Working standard solution was prepared by diluting 10 mL of stock solution to 100 mL with distilled water (100 μg / mL). 100 mg of the sample were weighed; the sugars extracted with hot 80% ethanol twice (5 mL of ethanol each time). The supernatant was collected and evaporated by keeping it on a water bath at 80°C. 10 mL of water was added to dissolve the sugars. An aliquot of 0.1 mL (V_1) was pipetted to separate test tubes. Different volumes of 0.2, 0.4, 0.6, 0.8 and 1 mL of the working standard solution were also pipetted out into a series of test tubes. The volume in both sample and standard tubes was made up to 2 mL with distilled water. Two millilitres distilled water were pipetted in to a separate tube to set a blank. 1 mL of alkaline copper tartrate reagent was added to each tube. All the tubes were placed in boiling water for 10 min. The tubes were cooled and 1 mL of arsenomolybdic acid reagent added to all the tubes. The volume in each tube was made up to 10 mL (V_2) with water. The absorbance of blue colour was read at 620 nm after 10 min. The concentration of the standards was adjusted if necessary to obtain OD values of the samples

closer to the standards' average and R^2 approximately one. A regression curve of optical densy was plotted against concentration (Figure 31).

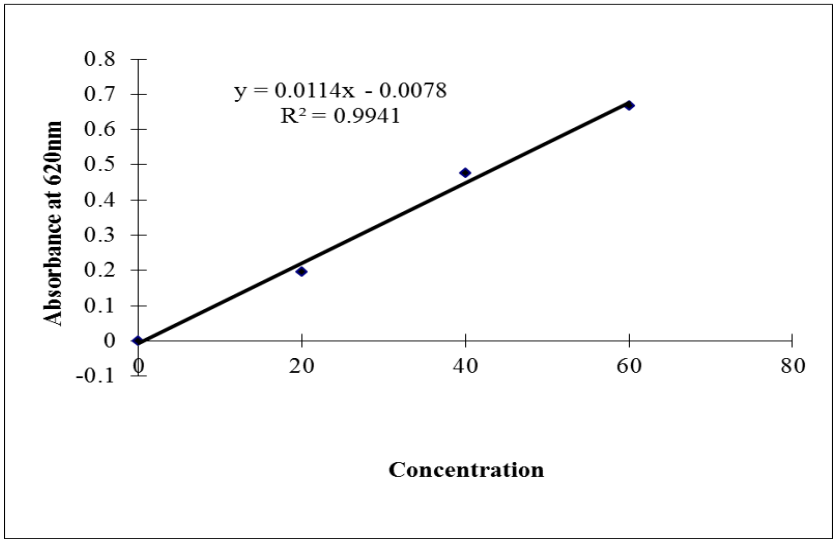


Figure 31: standard curve for concentration of reducing sugars standard solutions against OD values.

From the graph drawn, the amount of reducing sugars present in the sample was calculated as follows: Absorbance corresponding to 0.1 mL of test = x mg of glucose.
10 mL contains = x/ 0.1 × 10 mg of glucose = percentage of reducing sugars (Somogyi, 1952).
Percent reducing sugars = $Y/V_1 \times V_2$, where V_1 = aliquot volume and V_2 = final volume.

- Total free amino acids

Total amino acid content of okra accessions was determined by a formaldehyde titration method (Sorensen, 1907). Amino acids were extracted from 1 g of sample powder (Swt) by adding 20 mL 80% ethanol at 80°C for 30 min and shaking at 5 min intervals. The samples solutions were then mixed by vortex (Figure 32b), balanced, centrifuged at 10000 rpm for 10 min, filtered and the supernatant collected into 250 mL beakers. This extraction step was done four times and all supernatants collected, concentrated to 20 mL on water bath at 80°C. One hour before use, the pH of 37% formaldehyde solution was set at 8.4 by titration with 0.1N NaOH using a pH meter (Figure 32a), depending on the normality of NaOH available. Potassium hydrogen phthalate (KHP) was used to standardize 0.1N NaOH. About 0.5 g (Wt) of KHP was

pre-heated in a glass evaporator at 100°C for 1 h. The 0.5 g KHP was used to weigh four different samples of KHP close to 0.1 g in to 4 different flat bottom flasks. 75 mL of water boiled and cooled to about 40°C were added to each flask. The solution was mixed to dissolve and 2 to 3 drops of Phenolphthalein (PP) indicator were added to each flask. Each solution was titrated with 0.1N NaOH and the volume (V_1) of 0.1N NaOH required to change the colour to pink was used to calculate the normality of each solution. The average normality of the four solutions was used as the standard normality of 0.1N NaOH, which is closer to 0.1N.

Normality N_1 = Wt of KHP/Molecular weight KHP × 1000/V_1, where Wt = weight of KHP and V1 = volume of 0.1NNaoH required to change colour of KHP to pink.

Standard Normality of 0.1N NaOH = (N_1+ N_2 +N_3 + N_4)/4 = 0.095983.

The 0.095983N NaOH was used to titrate 37% formaldehyde solution and the sample solutions to pH 8.4. Ten mL 37% Formaldehyde solution (pH 8.4) were added to each sample solution (pH 8.4). The resultant solution (sample + formaldehyde) was titrated by 0.015N NaOH until pH is 8.4 (Figure 32a). The Normality (N) of 0.015N NaOH was prepared by making a 1:10 dilution of 0.1N (0.095983N) NaOH to get 0.009598N. The volume (V_2) of 0.015N NaOH needed to change the pH of the resultant solution to pH 8.4 was recorded and used to calculate the total amino acid content as follows: Total amino acid = (N × V_2 × 14×10^{-3} /Swt) 100%, where Swt = sample weight.

(a) (b)

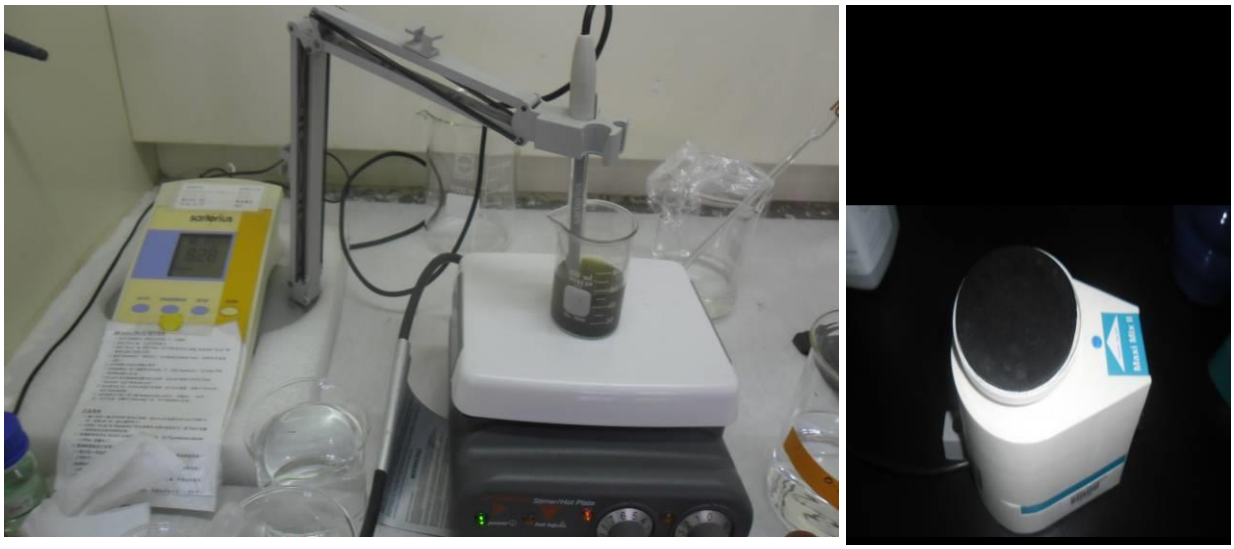


Figure 32: extration of free amini acids; a: pH meter and b: vortex machine (picture by Abang, 2012).

❖ Total phenols

The colorimetric method of Folin-Denis as described by Swain and Hillis (1959) was employed for the determination of phenolic compounds in the leaves. The procedure consists of extracting 200mg (Wt_1) dried powdered samples in 40 mL (V_1) methanol. The solutions were transferred into plastic tubes and weight balanced before centrifuging at 10000 rpm for 10 min. The supernatants were pipetted out into screw cap bottles and 1 mL (V_2) each taken and diluted with 2 mL methanol to make 3 mL (V_3) solution. From each 3-mL solution, 0.2 mL was taken for analysis of total phenols. The reagents used were anhydrous sodium carbonate saturated solution and Folin-Ciocalteu as phenol reagent. The normality of the phenol reagent, Folin-Ciocalteu was diluted from 2N to 1N with distilled water, and the sodium carbonate reagent solution was prepared by dissolving 35 g in distilled water to 100 mL mark. The standard solution was 1mM Chlorogenic acid and 17.7 mg (Wt_2) were diluted to 50 mL mark with methanol. From this standard solution, five different volumes (0, 0.2, 0.4, 0.6, and 0.8 mL) were placed in separate test tubes. The volumes were made up to 0.8 mL each by adding required volumes of methanol, to obtain five different concentrations (0, 0.25, 0.5, 0.75, and 1 mM) of the standard solution. From each concentration of the standard solutions, 0.2 mL was taken into separate test tubes for analysis. To the 0.2 mL of each sample solution and each standard solution, 3.2 mL distilled water were added, followed by 0.2 millilitre 1N phenol reagent and 0.4 mL of sodium carbonate saturated solution. The solutions were mixed and stored in the dark for 30 min. The spectrophotometer (Figure 33) was used to measure the OD values at 760 nm.

Figure 33: spectrophotometer for chromophore measurement (picture by Abang, 2012).

If necessary, the concentration of the standards was adjusted to obtain OD values of samples that are closer to the average standard and with R^2 approximately one. The standard curve below was used to determine the total phenolic content of the samples (Figure 34).

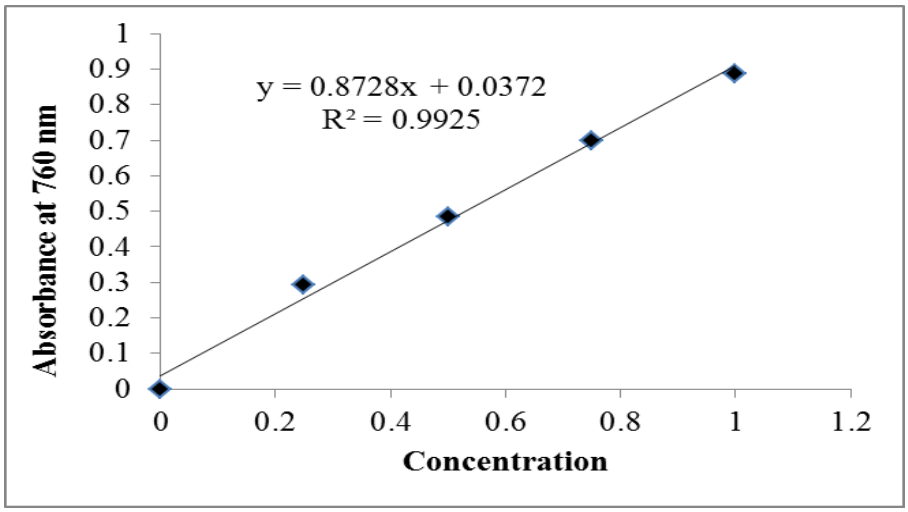


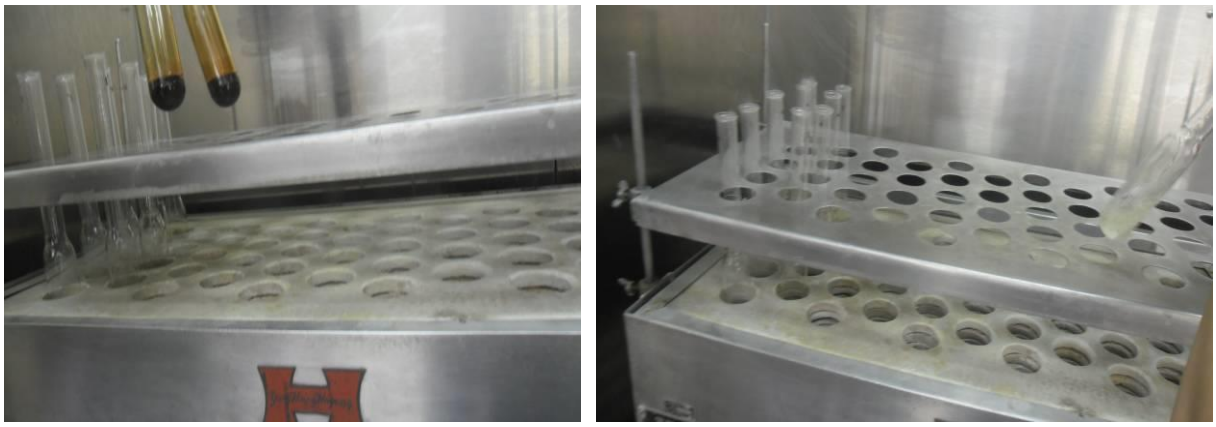
Figure 34: standard curve for concentration of standard solutions for total phenols against OD values.

Total phenols were calculated using the formular $Y = 0.8728(V_2/V_3)*OD - 0.0372$. Total phenols $= (Y*V_3*(Wt_1 + V_1)/Wt_1)*2Wt_2$, where V_1 = volume of methanol used to extract phenols, V_3 of supernatant used to collect subsample for analysis, Wt = sample weight.

- Total Nitrogen and Potassium content

Total leaf nitrogen content was determined following Kjeldahl's method as described by Bremner and Mulvaney (1982). The procedure consisted of digesting 0.2 g of sample powder with 5 mL concentrated H_2SO_4 in 50 mL-Kjeldahl digestion flask. The content was mixed and allowed to stand for 30 min. The flasks were then put into Al-block digestion furnace and heated with caution up to 300°C for 3 h. Materials adhering to the walls were dislodged and brought into contact with the acid by swirling the flasks at regular intervals. When all samples were dissolved to dark-brown colour solution (Figure 35a), the flasks were removed from the furnace and allowed to cool down to below 150°C. Two millilitres of Hydrogen peroxide (H_2O_2) were added and repeated until the solution changes to light yellow. The flasks were returned to the furnace

and heated continuously at 300°C until the solution was colourless (Figure 35b) and excess H_2O_2 decomposed.



(a) (b)

Figure 35: alluminium-block digestion furnace; a: dark-brown solution and b: colourless solution (picture by Abang, 2012).

The flasks were allowed to cool; 20 mL water were added and allowed to cool down again. The digest was diluted to 50 mL with distilled water and reserved for Nitrogen analysis. Four percent Boric acid-indicator solution was prepared and used as reagent. One hundred and sixty grams boric acid (H_3BO_3) was placed in a 4 L flask and 3.8 L water was added. The flask was heated while swirling until the boric acid is dissolved and the solution was cooled. A mixed indicator was prepared by dissolving 0.5 g of Bromocresol green and 0.1 g of Methyl Red in 100 mL of ethanol. To the cooled boric acid solution, 20 mL of mixed indicator were added, followed by 0.1 N NaOH with caution until the solution assumed a reddish purple tint (pH 4.2). The solution was made up to 4 L by adding water and mixed thoroughly before use. To do analysis of total Nitrogen, 5 mL of the digest were transferred to a distillation chamber and 5 mL of 10 N NaOH was added, and rinsed with 50 mL water. Distillation was commenced immediately by using 5 mL of H_3BO_3 indicator solution as receiver, and distilled until the distillate reached 60 mL. The distillate was titrated with 0.02 N H_2SO_4 to determine NH_4^+ - N.

Total Nitrogen (%) = [(Volume of H_2SO_4 – Blank) × 0.02 × F × 14 × D/ sample weight × 100]

Where, F = calibrating factor of 0.02N H_2SO_4, D = dilution factor, *e.g.*, take 5 mL from 50 mL digest, D = 50/5. The flame-photometer was used to analyze potassium content in the digested solution of plant tissues as described by Rayment and Lyons (2011).

II.3.3.2. Biophysical studies

The physical bases of resistance of the okra accessions were studied by evaluating the leaf trichome density, toughness and chlorophyll content.

- Leaf trichome density

For leaf trichome density, $1cm^2$ leaf pieces were collected from each side of the leaf main vein. The leaf pieces were mounted on a stereomicroscope and number of hairs was counted (Bourland *et al.*, 2003) (Figure 36).

Figure 36: leaf trichomes on abaxial surface of okra leaf (picture by Abang, 2012).

- Leaf toughness

Similarly, to evaluate leaf toughness, $1cm^2$ leaf pieces were also collected from each side of the main vein and mounted on a gram gauge (Figure 37). The gram gauge was designed by modifying a scale balance using a method described by Wheeler and Center (1997). A 0.52 mm diameter blunt probe was used to puncture each leaf that was placed on a measuring scale. The scale reading corresponding to the force required to puncture leaf tissues was recorded.

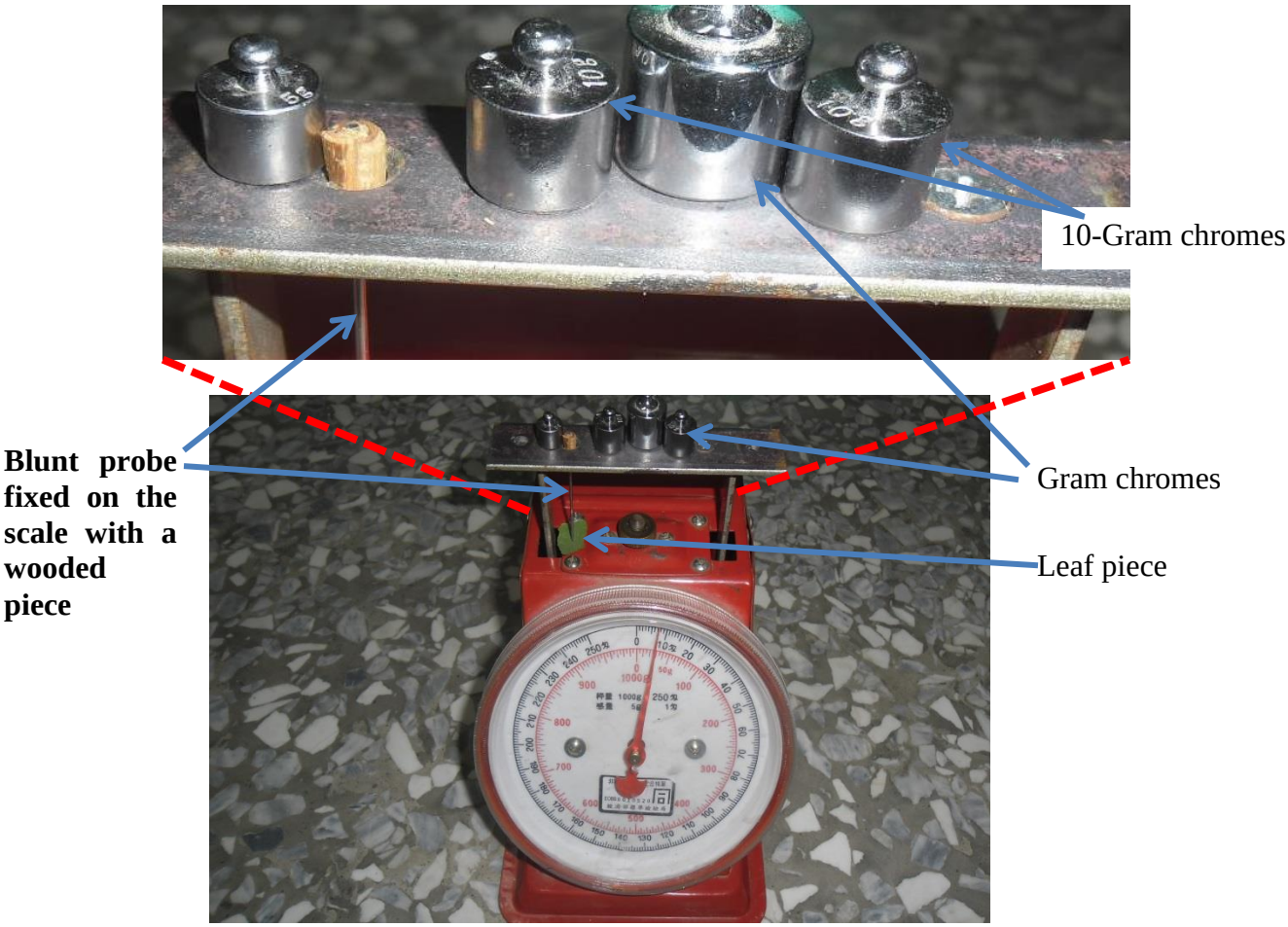


Figure 37: gram guage used for the measurement of leaf toughness (picture by Abang, 2012).

Measurements of leaf toughness and trichome densities were recorded for five replications, with two plants per replication.

- Leaf chlorophyll content

To determine total chlorophyll in leaf samples, 50 mg of each sample were macerated in 25 mL of 80% acetone to extract all chlorophyll pigments and the supernatant was centrifuged at 2000 rpm for 5minutes. Absorbance of supernatant was measured at the following wavelengths 534, 643 and 661 nm using the spectrophotometer (Lichttenathaler, 1987). Total chlorophyll, and chlorophyll a and b were expressed in microgram per gram (µg/g) fresh material.

II.3.4. Mechanisms of resistance

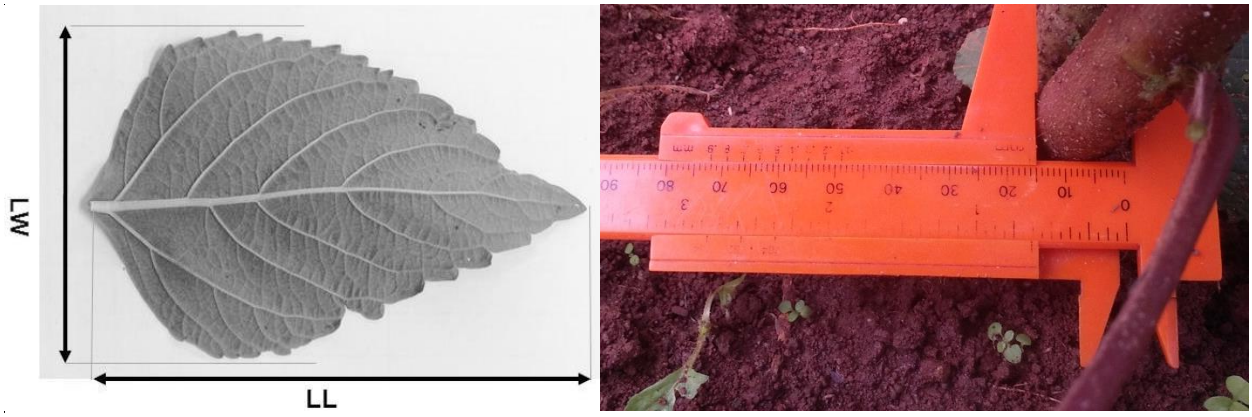
II.3.4.1. Tolerance

During the confirmatory screening trials, yield performance was evaluated by recording the following parameters as described by AVRDC (2001):

- days to flower, which is the numbers of days from sowing to when 50% of plants have at least one open flower (anthesis);
- days to commercial pod maturity, which is the numbers of days from sowing to the period when 50% of plants have at least one pod ready to harvest for consumption;
- number of pods per plant (recorded on harvest days);
- average pod weight (g/pod) recorded during harvest, pod length (cm) and width (cm) recorded from 10 fruits from the second harvest using a rular and a caliper respectively.

Plant parameters recorded for vigour were:

- leaf area (LW x LL) (cm^2), estimated at seedling, vegetative and reproductive stages;
- plant height (cm) and stem diameter at base (mm) average which are measures of five plants estimated at the end of plant growth using a rular and a caliper respectively.



(a) (Mack *et al.*, 2017) (b) (picture by Abang, 2014)

Figure 38: leaf area measurement (a) and stem diameter measurement with a caliper (b)
LW: Leaf width, LL: Leaf length

II.3.4.2. Antixenosis

The plants for studies of antixenosis were sown in plastic trays and single plant was transplanted at first true leaf stage in plastic pots (25 cm high and 20 cm diameter) containing

50% soil + 25% sand + 25% fowl manure (AVRDC, 2001). A pure colony of *A. gossypii* was maintained for two weeks on variety Clemson Spineless, a commercial cultivar of okra for Cameroon trial, and on accession PI249620 for studies in Taiwan. For non-choice conditions, the potted plants were placed individually in screen houses following complete randomized design (CRD). The settling behaviour of aphids on different okra accessions was determined to assess their preference for feeding and oviposition. The second fully expanded leaf from the apex of okra plants at 2 weeks after transplant (WAT), which is five-true-leaf stage (Figure 39a), were infested with ten adult aphids previously starved for two hours. After 72 h., adult aphids were counted to evaluate their permanence on the infested leaf. Plants with four or less adult aphids per infested leaf were considered non-preferred by aphids (Fereres, 1994). Under choice conditions, the test was conducted according to Martin and Fereres (2003). The bottoms of Petri dishes (diameter 85 mm) were covered with moist cotton and surface of cotton lined with filter paper (Figure 39b). At the edge of the paper, leaf-rectangles (2 × 1 cm) from the second fully expanded leaf of each accession at two weeks after transplant (2WAT) were placed. Then, 100 adult aphids after 2 h starvation were placed at the center of each Petri dish at equidistance from each leaf rectangles in a complete randomized design (CRD). The dishes were then transferred to a growth chamber at 25 ± 1ºC, 70 ± 10% RH and 12:12 h (L: D) photoperiod. The experiment was replicated five times with two plants per replication (ten-leaf rectangles per accession). The number of aphids located on each disk was counted at 48 h for the experiment with all accessions, and 72 h for the experiment with 1st selection.

a b

Figure 39: layout for non-preference test; a: no choice test and b: choice test (picture by Abang, 2014).

II.3.4.3. Antibiosis

Cotton aphids were collected from okra fields in July 2013, and placed on plants of the commercial cultivar (Gombo paysan), in cages for population establishment of pure colony of the cotton aphids. The plants for studies of antibiosis were sown in plastic trays and single ones transplanted at first true leaf stage in plastic pots (25 cm high and 20 cm diameter) containing 50% soil + 25% sand + 25% fowl manure (Figure 40 a). The potted plants were placed individually in mesh cages of 1 m high, 50 cm long and 50 cm wide (Figure 40 b). The mesh cages were arranged in net houses (Figure 40 c). To investigate whether the effect of antibiosis is systemic or localized at aphid feeding zone (Karban and Baldwin 1997), other potted plants were prepared in a similar manner but were previously infested with 25 to 35 aphids each at vegetative stage, and 100 to 200 aphids at reproductive stage. Five days later, the aphids were removed using a soapy solution prepared by mixing one teaspoon of liquid savon (TRI Shine Lime Dish Liquid) in 1 L water, in a spraying bottle. The solution was sprayed on okra leaves to kill and remove the aphids, and then leaves were rinsed with water. The plants were then used 24 h later for the life table study. The plants of the two experiments (plant previously infested and plants not previously infested with aphids) were arranged in CRD, consisting of three replications per genotype with 10 plants per replication.

(a) (b)

(c)

Figure 40: layout of antibiosis experiment; a: potted plants of different accessions under net cages; b: net houses with net cages and c: net houses as replications (picture by Abang, 2014).

Each plant was infested with five adult aphids on the second fully expanded leaf from the top at two weeks after transplanting. The adult cotton aphids were given 24 h to give birth then these adults and all their offsprings except one were monitored for life traits. This method is a modified version of cohort test described by Harris (1980). This method consists of leaving the leaves attached to the plants and observing only one aphid nymph, per cage, for moulting, and oviposistion, in a complete life table. Thus, excised leaves were not used because mechanical injury alone might induce changes in plant chemistry (Cipollini 1997). Greenhouse temperatures and relative humidity fluctuated in a daily cycle between 21.3 to 28°C and 79.1 to 100.6% during the first experiment with non-infested plant (Figure 41a); 22.2 to 29°C and 68.6 to 89.5% during

the second experiment with plant previously infested with aphids at Nkolbisson, Yaounde (Figure 41b).

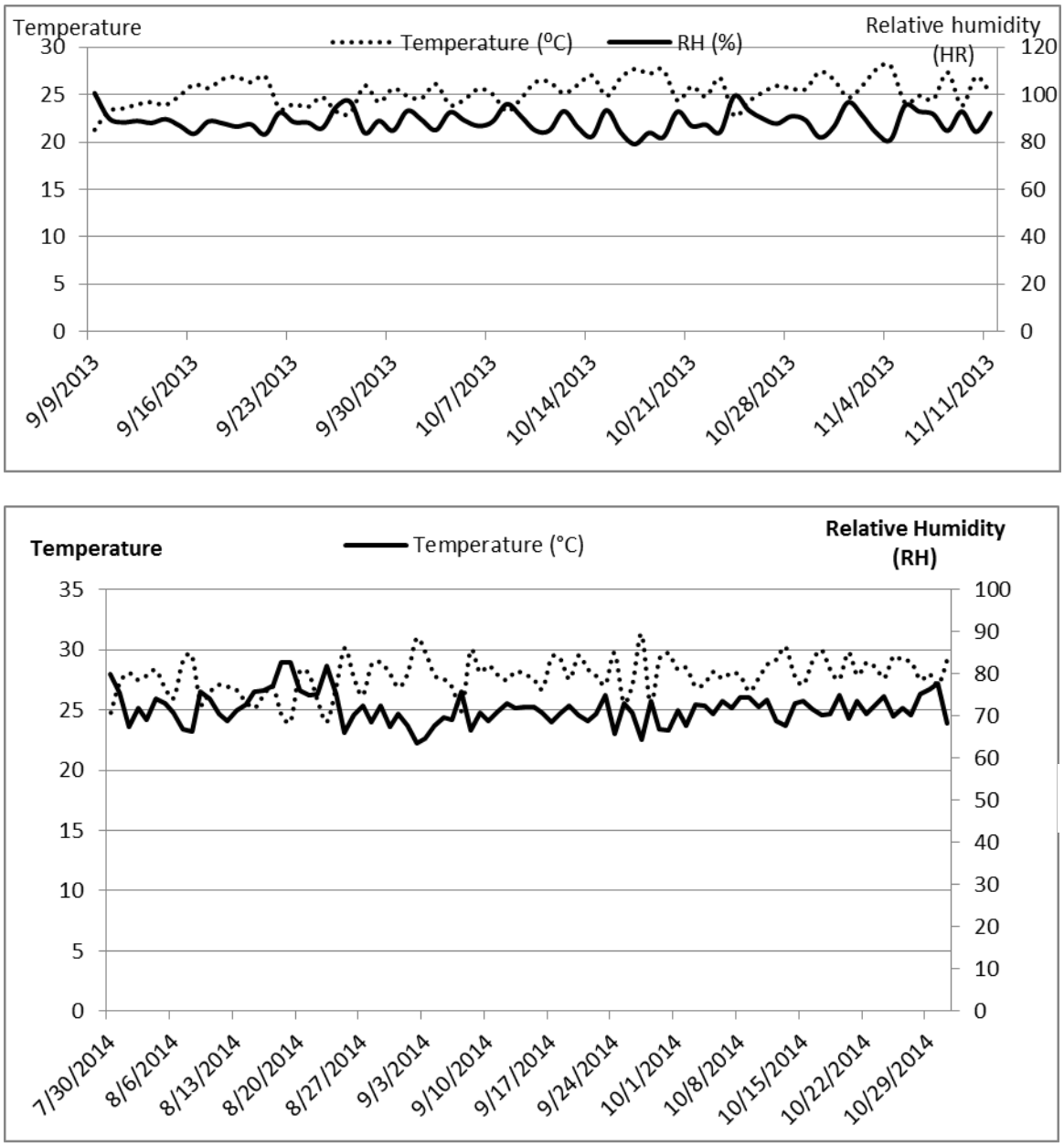


Figure 41: temperature and relative humidity in net houses; a: with plant not previousely infested and b: with previousely infested plants (summaries by Abang, 2014).

The exuviae were used to determine moulting time and number of immature stages; newborn nymphs were removed after counting. The following attributes of development and reproduction were studied: development time (DT) from the appearance of a nymph to its final moult, nymph mortality, moulting, instar development, reproductive time (RT) and the biological

cycle duration. Other parameters of reproduction were obtained by conducting age specific fertility life table.

To construct the age specific fertility life table, age specific survival rate and average aphids progeny in per age class were obtained. Based on these data, the intrinsic rate of natural increase, which is the number of aphids per aphid per day, was calculated by iteratively solving the equation of Birch (1948), $1 = \sum e^{-r_m \times x} l_x \times m_x$

where x is the age of the aphid in days, r_m is the intrinsic rate of natural increase, l_x is the age-specific survival, and m_x is the age-specific number of female offspring. Other parameters computed according to Carey (1993) were:

- the net reproductive rate ($R_0 = \Sigma\ l_x m_x$), which is the multiplication rate of an aphid per generation;
- the generation time from the appearance of a nymph to the time of onset of reproduction ($T_o = (\ln R_0) / r_m$);
- the population doubling time [$DT = (\ln 2) / r_m$] and;
- the finite rate of increase ($\lambda = e^{r_m}$), which is the number of aphids per aphid per unit time.

II.3.5. Aphid resistance and yield performance of okra accessions under various agro-ecological climates in Cameroon

Okra accessions identified as resistant to *Aphis gossypii* were screened in four of the five agro-ecological zones of Cameroon, to investigate ecological adaptation, resistance to aphids and yield performance. The trials were conducted during two seasons in 2014, from March to July and from August to December. The trial in Maroua, which is a semi-arid region, was conducted only once from September to December 2014. In each location, the local okra variety cultivated by farmers was included as checks. The common commercial variety in Cameroon "Gombo caféier" produced and supplied by a Cameroon-based seed company "Grenier du Monde Rural" (GMR), was also included in all locations. The trials were conducted using a randomized complete block design (RCBD) with three replications.

The trials were maintained following customary cultural practices, and without pesticide application to control aphids or other sucking insects. The trials were exposed to the natural infestation of aphids, and these insect populations were directly scored at bi-weekly intervals,

starting from four weeks after sowing (Figure 42). The crop management, screening methods and experimental design were similar to that of the confirmatory screening trial. Five plants from each accession were randomly selected and scored for aphids using a rating scale according to AVRDC (1979) as described in section II.3.2.1.

Figure 42: selected resistant accessions evaluated in Evodoula under farmer's environmental condition (picture by Abang, 2013).

The yield performance was evaluated by recording the following parameters: days to flower; days to commercial pod maturity; number of pods per plant (recorded on harvest days); average pod weight (g/pod); pod length (cm); pod width (cm) following AVRDC (2001), See II.3.4.1.

Plant parameters recorded for vigour were leaf area (cm^2), plant height (cm) and stem diameter at base (mm) according to AVRDC (2001), see II.3.4.1.

II.3.6. Data analysis

II.3.6.1. Data analysis for screening trials

Mean accession scores of aphid counts for each week during the preliminary, advanced replicated and advanced confirmatory screening trials were calculated across five plants. The

score data from each accession was expressed as the area under the infestation pressure curve (AUIPC), which is sum of weekly mean number of aphids per leaf, and was calculated using the following formula from Shaner and Finney (1977):

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

n: number of assessment times, Y: number of insects at time i

The AUIPC values for aphid population per leaf were subjected to a statistical analysis based on mean (m) and standard deviation (sd) (Table IV).

Table IV: rating for resistance where N = Total AUIPC for each accession, m = mean of number of aphids of all accessions (AVRDC, 1979)

AUIPC Range	Rating
N < (m–2sd)	Highly Resistant (HR)
(m–2sd) < N < (m–sd)	Resistant (R)
(m–sd) < N < (m)	Moderately Resistant (MR)
(m) < N < (m+sd)	Moderately Susceptible (MS)
(m+sd) < N < (m+2sd)	Susceptible (S)
N > (m+2sd)	Highly Susceptible (HS)

Other insect pests and natural enemies were also evaluated during the field trials. Damage due to leaf feeding beetle was recorded using the following score: 1 = no leaf damage, 2 = 1 to 25% damage, 3 = 26 to 50% damage, 4 = 51 to 75% damage, 5 = >75% damage (Banful and Mochiah, 2012). The data for pest, yield and plant parameters were subjected to analysis of variance (ANOVA) with one-way procedure of SAS, version 9.2 (SAS Institute, Cary, NC, U.S.A.). Tukey's HSD Test was used to separate the means at 5% significance level of probability.

II.3.6.2. Data analysis for biophysical and biochemical bases of resistance

Data obtained from experiments on the biochemical and biophysical properties of accessions were subjected to analysis of variance (ANOVA) with one-way procedure of SAS, version 9.1 (SAS Institute, Cary, NC, U.S.A.). Least significant difference (LSD) test was used to separate the means at 5% significance level of probability.

II.3.6.3. Data analysis for mechanismss of resistance

The choice test on the antixenosis and settling behaviour of aphids was analysed using Kruskal-Wallis test with one-way ANOVA a non-parametric test. Where significant differences were found ($P < 0.05$) between treatments, Tukey's HSD test was used as *post hoc* procedure for mean comparison. For antibiosis and tolerance respectively, values of the development and reproductive performance of *Aphis gossypii* and yield and plant parameters were subjected to analysis of variance (ANOVA) with one-way procedure of SAS, version 9.1 (SAS Institute, Cary, NC, U.S.A.). Tukey's HSD Test was used to separate the means at 5% significance level of probability.

II.3.6.4. Data analysis for multilocation trials

The scored data for aphid count from each accession of the multilocation experiments were analysed as in the screening trials.

Yield and plant data were subjected to analysis of variance (ANOVA) with one-way procedure of SAS, version 9.2 (SAS Institute, Cary, NC, U.S.A.). Tukey's HSD Test was used to separate the means at 5% significance level of probability.

CHAPTER III: RESULTS AND DISCUSSIONS

III.1. Results

III.1.1. Genetic studies of aphid populations from Taiwan and Cameroon

The genetic studies on six aphid populations collected from Cameroon (3 populations from Yaoundé and 1 population from North-West Region) and Asia (1 from India and one fron Taiwan) showed that, there were no biotypes of *Aphis gossypii* between the four locations. The primers (Ago_CoxI_f1and r1.1, Ago_CoxI_f1and r1.2, Ago_CoxI_f2 and r2.2) produced a PCR product of 750 bp, while only Ago_CoxI_f2 and r2.1 produced a PCR product of 900 bp (Figure 43). With the primers used for sequencing, the PCR product was approximate 900 base pairs. The sequences have been submitted in the NCBI GenBank and the accessions numbers are as follows: Cameroon [High altitude savannah population (KF385392), farmer okra field in warm and humid forest (KF385393), Hibiscus plant in warm and humid forest (KF385394), on-station okra at warm and humid forest (KF385395)], Taiwan (KF385396) and India (KF385397). The nucleotide sequence comparison showed 100% similarity between the six populations because of sequence homogeneity (Figure 44). The phylogenetic analysis also confirmed that the six aphid populations from Cameroon, India and Taiwan are genetically identical. *Aphis craccivora* was used as an out-group and the nucleotide sequence comparison showed 93% similarity with *A. gossypii*. The numbers (1 to 6) represent the six aphid populations with each primer pair; M is the control lane, which is DNA marker lane.

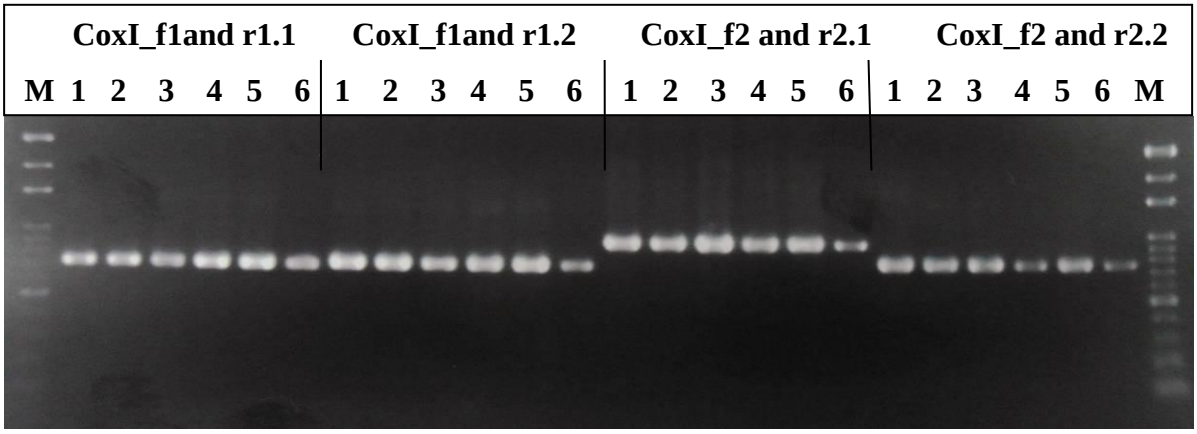


Figure 43: gel images of amplicons for six populations of *A. gossypii* (picture by Abang, 2012).

Figure 44: phylogenetic analysis between DNA of six populations of *Aphis gossypii* from Cameroon, India and Taiwan and that of *Aphis craccivora* (analysis by Abang, 2012).

III.1.2. Preliminary screening to identify the accessions resistant to *Aphis gossypii*

In 2011, out of the 88 accessions screened from March to May, eight accessions (VI051114, VI050958, VI059164, VI036213, VI046559, VI050960, VI033805, and VI056457) were rated as resistant to aphid infestation in Taiwan (Appendix 1). In a subsequent preliminary screening from September to November 2011(autumn) in Taiwan, 54 of 68 accessions were rated as moderately resistant, and none as either resistant or highly resistant (Appendix 2). Only four (VI058525, VI060313, VI058519, VI058521) with the least infestation out of the 54, were selected for advanced replicated trial because we wanted to limit the number of accessions. During the preliminary screening period in Cameroon, from November 2011 to February 2012, 10 resistant accessions (VI060809,VI060810, VI060858, VI060702, VI060787, VI060740, Evodoula, VI060704, VI060784, VI060786) were identified as resistant out of 115 (Appendix 3).

In 2012, out of 96 accessions screened from March to July (spring), 66 were rated as moderately resistant and none as resistant to aphid infestation in Taiwan (Appendix 4). Among the 66, 22 had no infestation, but only four (VI044242, VI033796, VI033824 and VI046537) out of 22 were selected for advanced replicated trials because of lack of seeds. In a subsequent preliminary screening in autumn 2012 (September to November), 8 out of 64 accessions were rated as resistant to aphid infestation in Taiwan (Appendix 5), but only 7 (VI039614, VI060688, VI060794, VI060817, VI060818, VI060866, VI041210) were used in advanced replicated trial because one (VI049964) had no seeds.

III.1.3. Advanced replicated screening of accessions resistant to *Aphis gossypii*

III.1.3.1 First selection (2011)

The 22 okra accessions obtained from the first three preliminary screening trials in 2011 were evaluated in advanced screening. However, only 19 accessions (VI060740, VI060784, VI060787, VI060809, VI060810, VI060858 VI060313, VI058525, VI058521, VI058519, VI050960, VI050958, VI059164, VI056457, VI046559, VI051114, VI036213, VI033805 and variety Evodoula) were screened in the advanced screening trial; seeds of the remaining three lines did not germinate (Table V). Out of 19 accessions screened in the first advanced replicated screening, three (VI051114, VI036213 and VI033805) were rated as resistant to aphids; they are all *Abelmoschus esculentus*, whereas in Cameroon *Ab. caillei* is the most cultivated species. More than 20 accessions of *Ab. caillei* were screened during the present study, which included two commercial varieties (Gombo caféier and Gombo paysan). All *Ab. caillei* accessions screened were more susceptible to aphids in Cameroon than *Ab. esculentus* accessions.

Table V: first advanced replicated screening in Yaoundé, Cameroon, March to June 2012, of the 19 okra accessions identified as resistant from the first three preliminary screenings

Accessions	Origin	AUIPC (mean number of aphids per leaf)	Resistance/Susceptibility
VI060313	Tanzania	572.0	Highly susceptible
VI050960	Zambia	421.8	Susceptible
VI058521	Unknown	363.0	Moderately susceptible
VI060740	Unknown	339.4	Moderately susceptible
VI058525	Unknown	335.5	Moderately susceptible
VI050958	Zambia	324.7	Moderately susceptible
VI060810	Turkey	320.6	Moderately susceptible
VI060784	USA	314.7	Moderately susceptible
VI059164	Unknown	300.6	Moderately susceptible
Evodoula	Cameroon	253.7	Moderately resistant
VI058519	Unknown	229.8	Moderately resistant
VI060787	USA	224.9	Moderately resistant
VI060809	Turkey	221.6	Moderately resistant
VI056457	Yugoslavia	207.8	Moderately resistant
VI060858	Mali	202.4	Moderately resistant
VI046559	Thailand	174.5	Moderately resistant
VI051114	Philippines	157.8	Resistant
VI036213	Philippines	134.7	Resistant
VI033805	Philippines	87.9	Resistant
Overall mean (m)		273.02	
Standard deviation (S.D.)		112.59	

III.1.3.2 Second selection (2012)

In the second advanced replicated screening conducted in Cameroon, fifteen varieties were evaluated; eleven collected from farmers' fields in different locations, and four identified as resistant from the fourth preliminary screening during spring 2012 in Taiwan. Out of the 15 accessions, only one (VI033824) was rated as resistant, whereas ten varieties were moderately resistant (Table VI). None of the farmers' varieties was resistant.

Table VI: second advanced replicated screening, from October 2012 to March 2013 in Yaoundé, of okra varieties from farmers fields and resistant accessions from fourth preliminary trial

Accession	Origin	AUIPC (N) (mean number of aphids per	Resistance/ Susceptibility	Species
VI033824	Philippines	474.3	Resistant	*Abelmoschus esculentus*
VI033796	Malaysia	516.0	Moderately resistant	*Abelmoschus esculentus*
Bityili giant	Cameroon	544.4	Moderately resistant	*Abelmoschus caillei*
Njombe caffeier	Cameroon	551.1	Moderately resistant	*Abelmoschus caillei*
Maroua	Cameroon	582.3	Moderately resistant	*Abelmoschus esculentus*
VI033778	Malaysia	671.0	Moderately resistant	*Abelmoschus esculentus*
VI057245	Cambodia	684.1	Moderately resistant	*Abelmoschus esculentus*
Small Soppo	Cameroon	696.5	Moderately resistant	*Abelmoschus caillei*
Evodoula six months	Cameroon	705.4	Moderately resistant	*Abelmoschus caillei*
Munya Buea	Cameroon	755.9	Moderately resistant	*Abelmoschus caillei*
VI046537	Thailand	763.4	Moderately resistant	*Abelmoschus esculentus*
Baba I	Cameroon	785.5	Moderately susceptible	*Abelmoschus caillei*
Njombe red	Cameroon	929.1	Moderately susceptible	*Abelmoschus caillei*
Ebebda green	Cameroon	1015.5	Moderately susceptible	*Abelmoschus caillei*
Njombe green	Cameroon	1219.2	Moderately susceptible	*Abelmoschus caillei*
Babungo	Cameroon	1519.8	Highly susceptible	*Abelmoschus caillei*
Overall mean (m)		781.96		
Standard deviation		287.02		

The present results also showed significant difference in productivity among the varieties $F_{(15, 25\ d\ f)} = 5.09$ and $P < 0.0002$) (Figure 45). The average number of pods in farmers' varieties and *Abelmoschus caillei* species were higher (5 to 13 pods per plant) than AVRDC accessions

and *Abelmoschus esculentus* species (1 to 4 pods per plant) (Figure 45). The most susceptible accession (Babungo) produced the highest yield (13 pods per plant). Susceptible accessions yielded higher than resistant accessions (Figure 45).

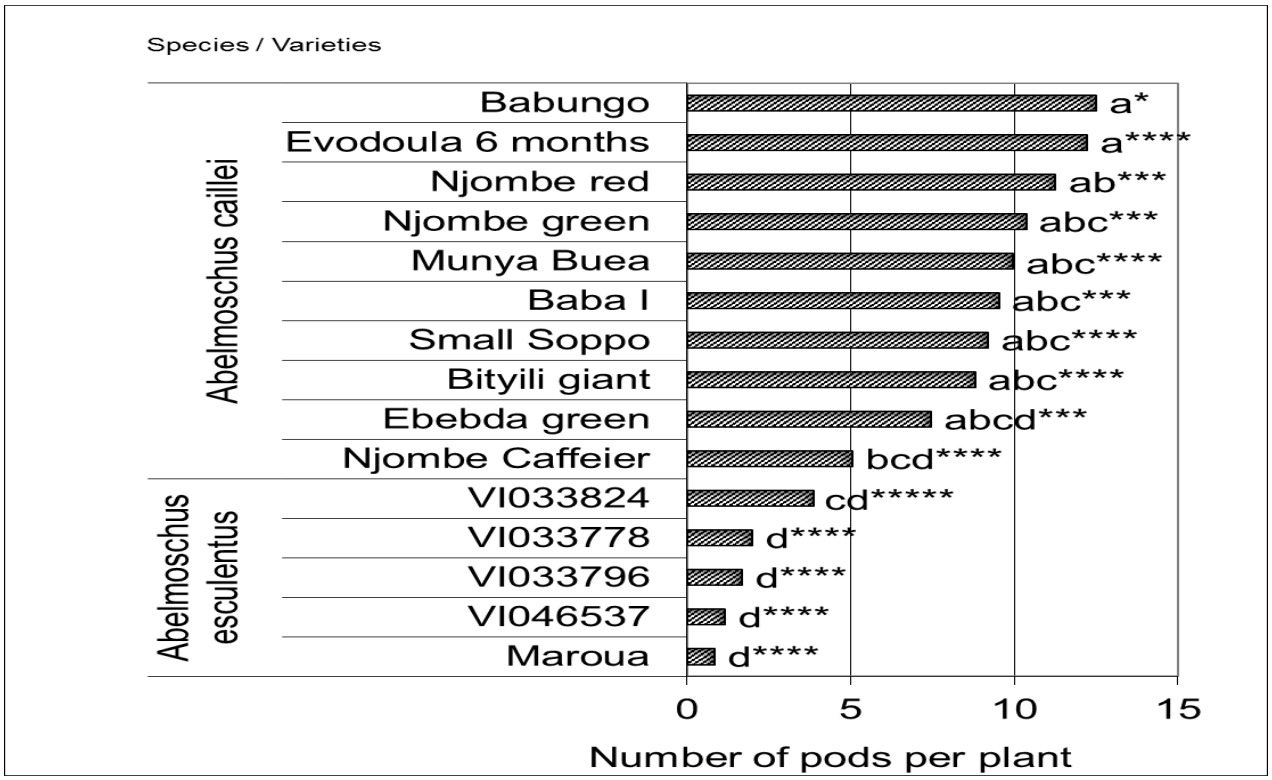


Figure 45: okra yield of 11 farmers and 4 aphid-resistant accessions during second advanced replicated screening from October 2012 to March 2013 in Yaoundé, *highly susceptible, **susceptible, ***moderately susceptible, ****moderately resistant, *****resistant.
Mean values respresented by bars with different letters are significantly different ($P < 0.05$) following Duncan's multiple range test.

The results showed significant differences among varieties in days to 50% anthesis ($F = 8.58$ and $P < 0.0001$) and in days to 50% commercial maturity ($F = 12.92$ and $P < 0.0001$). Most farmers' varieties were late flowering (60 to 100 days to anthesis) but three of the AVRDC accessions (VI033796, VI033824, and VI033778 including the farmer's variety from Maroua) were early flowering (52 to 54 days to anthesis) (Figure 46). All farmers' varieties, except the one from Maroua, were *Ab. caillei* with large epicalyx, while all four AVRDC accessions were *Ab. esculentus* with spiny epicalyx. Days to 50% commercial maturity followed a similar trend as

days to 50% anthesis. The former parameter for farmers' varieties, range from 67 to 110 days and from 60 to 80 days for the AVRDC accessions, and the one from Maroua (Figure 46). *Ab. caillei* flowers late (60 to100 days) whereas *Ab. esculentus* flowers early (52 to 54 days). A similar trend was observed for the two species in terms of days to commercial maturity from 60 to 80 days in *Ab. esculentus* and 70 to 110 days in *Ab. caillei* before harvesting is started on 50% of the plants. Three varieties of *Ab. esculentus* stopped production at three months, while two of them were able to reach four months. All the *Ab. caillei* varieties extended their production period to about four and a half-month.

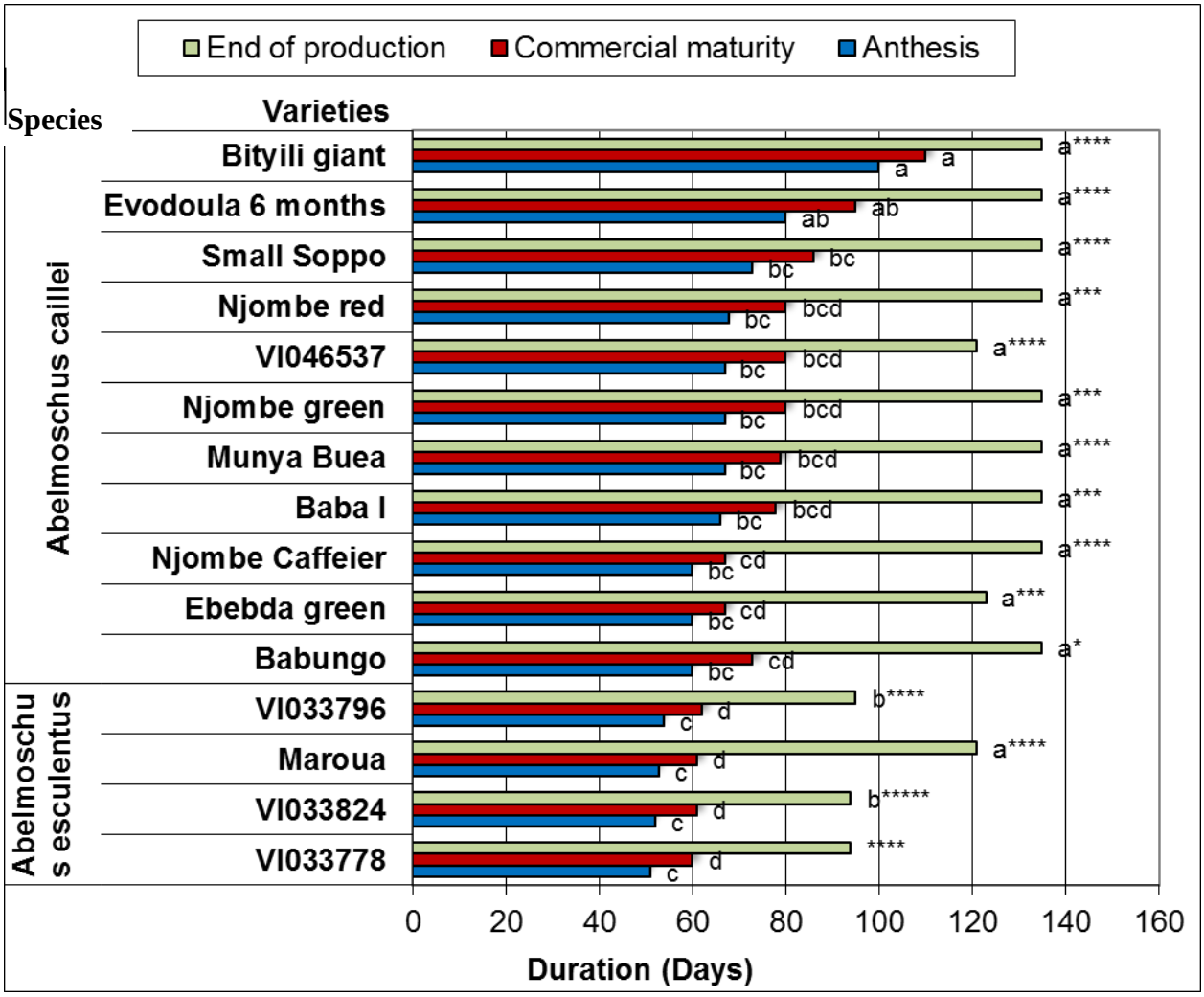


Figure 46: phenological durations of the 15 okra accessions in days to flowering, commercial maturity and end of production evaluated in Yaoundé from October 2012 to March 2013, *****resistant, ****moderately resistant, ***moderately susceptible, **susceptible, *highly susceptible.

For the same coloured bars, mean values with different letters are significantly different ($P < 0.05$) following Duncan's multiple range test.

III.1.3.3 Third selection (2013)

The seven accessions selected as resistant from the preliminary experiment during autumn 2012 in Taiwan were screened in third advanced replicated trial in 2013; five accessions were moderately resistant and none was resistant (Table VII).

Table VII: third advanced replication screening from March to July 2013 in Yaoundé, Cameroon

Accession	Origin	AUIPC (N) (number of aphids)	Resistance/Susceptibility
VI060818	Mali	594.9	Moderately resistant
VI060794	Côte d'Ivoire	625.7	Moderately resistant
VI060688	India	640.5	Moderately resistant
VI041210	Philippines	656.2	Moderately resistant
VI039614	Bangladesh	808.4	Moderately resistant
VI060866	Mali	1015.3	Moderately susceptible
VI060817	Brazil	1415.7	Susceptible
Mean (m)		822.4	
Standard deviation		300.2	

During this study, aphids were generally the most important pest of okra in terms of numbers as seen during the unique preliminary trial conducted in Cameroon from November 2011 to February 2012, and in two of the advanced replicated trials from March to July 2012 and 2013. The results showed that the number of the most important pests were lowest on the same accessions (Appendix 6, 7 and 8). The second most important pest was leaf-feeding beetles followed by white fly. However, the damage due to leaf beetle ranged from score 2 to 4 with the dominant score being three (3) (25 to 50% leaf damage). The confirmatory screening trials were focused only on the most important pest observed which *Aphis gossypii* was.

III.1.4. Confirmatory screening

Results showed that the farmers' variety (Kirikou) was generally more susceptible to aphids than most of the selected accessions and the commercial variety Gombo caféier, except VI051114. Out of 12 accessions, one (VI041210) was resistant during the first season from March to July in Cameroon and during autumn from October to January in Taiwan. VI057245 and Gombo caféier were resistant during the second season from September to December but moderately during the first in Cameroon, while seven were moderately resistant during the first

season and three during the second season. VI060794 and Kirikou were the most susceptible accessions; VI051114 and VI060818 were also suceptible at least once. Generally, the most resistant accessions were VI041210, Gombo caféier and VI057245 (Table VIII).

Table VIII: confirmatory screening of accessions selected as resistant or moderately resistant from the three advanced replicated trials including three susceptible checks (two from Cameroon and one from Taiwan)

Accession	Origin	Area Under Infestation Pressure Curve (Aphid per leaf)		
		Autumn 2013 in Taiwan	First season 2014 in Cameroon	Second season 2014 in Cameroon
VI041210	Pilippine	42.9 R	39.8 R	59.8 MS
VI057245	Cambodia	43.5 R	52.9 MR	49.7 R
Gombo caféier	Cameroon		49.2 MR	50.6 R
VI033824	Philippines	49.8 MR	49.7 MR	63.8 MS
VI060688	India	58.5 MS	47.5 MR	57.9 MR
VI033805	Philippines	52.9 MR	56.8 MS	62.2 MS
VI060818	Mali	51.8 MR	66.4 S	54.8 MR
VI036213	Philippines	66.5 MS	49.6 MR	57.7 MR
VI039614	Bangladesh	67.1 MS	50.9 MR	60.4 MS
Kiikou	Cameroon		55.4 MS	68.0 S
VI051114	Philippines	62.2 MS	68.3 S	59.8 MS
VI060794	Côte d'Ivoire	82.8 HS	50.1 MR	66.5 S
Mean (m)		57.8	53	59.3
S.D.		12.3	7.91	5.6

"Blank space indicates that the variety was not tested in that country.
HR = Highly resistant, R= Resistant, MR = Moderately resistant, MS = Moderately susceptible, S = Susceptible, HS = Highly susceptible.

III.1.5. Biochemical and biophysical characteristics that play a role in resistance

III.1.5.1. Biochemical characteristics

❖ Constitutive biochemical analysis

Plant metabolites studied to elucidate the bases of resistance were secondary metabolites such as tannins, total phenols and primary metabolites such as free amino acids, total sugars and total nitrogen. There was no significant difference among the accessions in total sugar and tannin contents (P = 0.55 and P = 0.37 respectively) (Table IX). There were significant differences among the accessions for free amino acids (P = 0.03), total phenols (P = 0.005) and total nitrogen (P = 0.0001). VI057245 that was selected as susceptible check was among the most resistant

accessions in confirmatory screening. VI057245 and VI051114 had significantly higher leaf phenols than VI033805 and VI036213. The susceptible check VI057245 had higher amounts of free amino acids (not significantly), total nitrogen and total phenols than VI033805 and VI036213 (Table IX).

Table IX: okra leaf content of phytochemicals conducted and analysed in Taiwan in 2012

Accession	Nitrogen (%)	Phenols (mg/100 g)	Tannins (mg/g)	Total Sugars (mg/g)	Reducing sugars (%)	Free amino acids (%)
VI057245	2.75a	90.2a	0.7	1.8	12.8	0.09ab
VI033805	1.85b	70.4b	0.7	1.6	12.6	0.08b
VI051114	2.43a	81.4a	0.7	1.6	13.2	0.10a
VI036213	1.95b	78.2b	0.7	1.5	10.1	0.07b
F-Value	15.05	9.8	1.1	0.74	1.04	3.85
P-Value	0.0001	0.005	0.37	0.55	0.43	0.03

Mean values with different letters in a column are significantly different at $P < 0.05$.

❖ Aphid feeding induced biochemical analysis at different crop phenologies

Biochemical studies on the first (2011) and second (2012) selections showed that, at vegetative stage, six weeks after sowing (WAS) there were significant differences among accessions in level of total nitrogen, potassium, tannins and total sugars contents in plants with and without aphid infestation. The local check (Kirikou) and VI033805 which were more susceptible during the confirmatory screening, had significantly lower tannins following aphid infestation than the other three less susceptible accessions (VI051114, Gombo caféier, VI033824), but not significantly different from VI036213 (Table X). In addition, VI033805 had significantly higher total sugars contents; but in Gombo caféier, one of the most resistant varieties, presented in Table VIII became significantly lower following attack.

At reproductive stage (10 WAS) there were significant differences among accessions, only in tannins and total sugars, and in nitrogen with plant previousely infested with aphids. Again, Kirikou and VI033805 had the lowest amount of tannins after inferstation, but only significantly different from Gombo caféier. While the amount of tannins decreased when plants were infested in the susceptible farmer check (Kirikou), it was increasing in the resistant accessions. Total sugars were also significantly higher in susceptible Kirikou while reducing sugars increased by more than 100%, Potassium content decreased after infestation more than in the resistant accessions (Table XI).

Table X: biochemical contents of okra for first (2011) and second (2012) selections with and without aphids (at 6 weeks after sowing)

Phytochemical	With or without aphids	VI051114	Gombo caféier	Kirikou	VI033824	VI033805	VI036213	$F_{(5,12df)}$-Value	P-Value
Phenols (mg/100 g)	Without	1868	1759	1409	1272	1227	1198	*1.67*	*0.217*
	With	1473	1310	1328	1324	1233	1313	*0.82*	*0.558*
Tannins (mg/g)	Without	0.40b	0.51a	0.41ab	0.43ab	0.40b	0.42ab	*3.49*	*0.035*
	With	0.46a	0.44a	0.37b	0.45a	0.37b	0.42ab	*3.72*	*0.029*
Total Sugars (mg/g)	Without	2.7ab	0.9c	2.0bc	3.3ab	3.7a	2.1abc	*8.31*	*0.001*
	With	3.2a	1.3b	1.3b	3.2a	3.2a	3.2a	*7478*	*<.0001*
Reducing sugars (%)	Without	79.2	59.9	28.9	46.9	59.0	49.4	*1.37*	*0.300*
	With	29.9	40.2	37.0	37.6	23.1	34.5	*2.78*	*0.068*
Total Nitrogen (%)	Without	6.5ab	6.6a	4.1c	5.6b	5.7b	6.9a	*9.03*	*0.0018*
	With	7.0a	7.4a	4.4c	6.6a	5.1bc	6.2ab	*8.65*	*0.0021*
Total Potassium (%)	Without	2.7b	2.7b	3.6a	2.7b	*3.5a*	2.6b	*11.3*	*0.0007*
	With	2.4c	2.8b	3.0b	2.5bc	3.6a	2.3c	*14.97*	*0.0002*

Mean values with different letter(s) in a row are significantly different at $P < 0.05$.

Table XI: biochemical contents of okra for 2011 and 2012 selections with and without aphids (reproductive stage at 10 weeks after sowing)

Phytochemicals	With or without aphids	VI051114	Gombo caféier	Kirikou	VI033824	VI033805	VI036213	$F_{(5,12df)}$-Value	P-Value
Phenols (mg/100 g)	Without	1299	1396	1390	*1641*	*1757*	*1425*	*2.94*	*0.058*
	With	1367	1243	1194	*1345*	*1266*	*1255*	*2.76*	*0.069*
Tannins (mg/g)	Without	0.54ab	0.52ab	0.64°	0.47b	0.43b	0.50b	*6.40*	*0.004*
	With	0.66ab	0.69a	0.55ab	0.64ab	*0.53b*	0.57ab	*3.84*	*0.026*
Total Sugars (mg/g)	Without	1.31ab	*1.31ab*	1.33°	1.31ab	1.30b	1.30b	*4.22*	*0.019*
	With	1.32b	*2.56a*	1.32b	1.32b	1.32b	1.31b	*4.01*	*0.023*
Reducing sugars (%)	Without	48.0	32.3	*35.3*	34.4	43.9	24.7	*1.78*	*0.192*
	With	53.5	57.8	*78.6*	63.2	49.5	52.4	*0.36*	*0.868*
Nitrogen (%)	Without	5.6	5.1	4.6	5.5	4.6	5.4	*2.14*	*0.152*
	With	5.95a	5.60a	3.59b	5.21a	5.27a	5.83a	*4.34*	*0.02*
Potassium (%)	Without	2.8	3.3	4.0	3.1	3.2	3.8	*1.38*	*0.316*
	With	2.7	3.7	2.9	2.7	3.2	3.2	*1.07*	*0.428*

Mean values with different letter(s) in a row are significantly different at $P < 0.05$.

In 2013, biochemical studies of selected okra accessions at six weeks after sowing showed that there were significant differences among them in total phenols ($P = 0.022$) and total tannins ($P = 0.017$) in plants without aphids, but not in plants infested with aphids. VI057245 that was the most resistant had significantly higher phenol content. Although tannins were lower in VI057245, they increased after attack while they were decreasing in the other less resistant ones. Plant reducing sugars and total nitrogen showed significant differences among accessions only when infested with aphids. The most susceptible accession, VI060794 had significantly higher total nitrogen than the two most resistant ones (VI057245 and VI041210). There were very significant differences among accessions with and without aphid infestation in Potassium content, though only VI057245 had significantly higher Potassium content than most one (Table XII).

At 10 weeks after sowing, there were significant differences in total phenols, only for plants infested with aphids ($P = 0.036$) (Table XIII). For tannins, significant differences were found in both plants infested with ($P = 0.009$) and without aphids ($P = 0.007$). Total tannins content increased in all accessions after infestation but the increased was more in the two most resistant ones (VI057245 and VI041210). Total sugars and reducing sugars also showed significant differences with ($P = 0.025$ and $P < 0.0001$ respectively) and without aphids ($P = 0.0003$ and $P = 0.0096$ respectively). Total nitrogen showed significant difference only without aphids ($P = < 0.0001$), with the susceptible accession (VI060794) having significantly higher total nitrogen than the other ones. Contrary to expectations, potassium content was higher in the susceptible VI060794, though also as expected, it was significantly higher in the two most resistant VI057245 and VI041210 than in the other moderately resistant one (VI039614, VI033824, VI060688 and VI060818), when infested with or without aphids.

Table XII: biochemical content of okra for third (2013) selection with and without aphids (vegetative growth at 6 weeks after sowing)

Phytochemical	With or without aphids	VI060818	VI057245	VI060794	VI060688	VI041210	VI039614	VI033824	$F_{(6,14\ df)}$ -value	P-value
Phenols (mg/100 g)	Without	1142ab	1436a	1229ab	1173ab	1073b	1090b	1072b	*3.64*	*0.022*
	With	1537	1432	1508	1461	1355	1442	1645	*0.50*	*0.795*
Tannins (mg/g)	Without	0.56ab	0.52b	0.62a	0.62a	0.60ab	0.55ab	0.58ab	*3.92*	*0.017*
	With	0.53	0.64	0.56	0.54	0.49	0.50	0.55	*1.18*	*0.372*
Total sugars (mg/g)	Without	1.29	1.30	1.29	1.30	1.31	1.30	1.31	*2.32*	*0.091*
	With	1.30	1.31	1.30	1.30	1.30	1.30	1.30	*0.78*	*0.597*
Reducing sugars (%)	Without	23.2	27.9	16.6	19.5	26.5	22.7	21.0	*1.97*	*0.138*
	With	18.9c	42.1ab	22.7c	24.9bc	35.5abc	46.8a	24.2bc	*3.40*	*0.028*
Nitrogen (%)	Without	3.9	3.5	4.1	4.0	3.8	3.9	4.3	*2.5*	*0.074*
	With	4.1a	3.4b	4.0a	3.8ab	3.4b	3.8ab	4.1a	*8.16*	*0.0006*
Potassium (%)	Without	2.16b	2.73a	2.79a	2.00b	2.59a	1.95b	2.17b	*22.78*	*<0.0001*
	With	2.12c	2.46a	2.33ab	1.69e	2.21bc	1.81de	1.86d	*32.07*	*<0.0001*

Mean values with different letter(s) in a row are significantly different at $P < 0.05$.

Table XIII: biochemical content of okra for third (2013) selection with and without aphids (reproductive growth at 10 weeks after sowing)

Phytochemical	With or without aphids	VI060818	VI057245	VI060794	VI060688	VI041210	VI039614	VI033824	$F_{(6,14\ df)}$ -value	P-value
Phenols (mg/100 g)	Without	1866	1633	1624	1585	1564	1359	1264	*1.70*	*0.194*
	With	1481a	1564b	2171a	2117a	1537b	1765ab	1602b	*3.14*	*0.036*
Tannins (mg/g)	Without	0.43a	0.36ab	0.44a	0.40ab	0.33b	0.40ab	0.40ab	*4.81*	*0.007*
	With	0.47ab	0.42b	0.46ab	0.43b	0.46ab	0.47ab	0.49a	*4.53*	*0.009*
Total Sugars (mg/g)	Without	1.30b	1.30b	1.29b	1.29b	1.29b	1.30ab	1.32a	*9.22*	*0.0003*
	With	1.29ab	1.30ab	1.29b	1.29ab	1.29b	1.30ab	1.31a	*3.50*	*0.025*
Reducing sugars (%)	Without	37.6b	38.8ab	18.0c	37.0b	*26.2bc*	38.4ab	*55.9a*	*4.50*	*0.0096*
	With	24.2cd	38.1bc	15.0d	27.6cd	*29.7bc*	42.3b	*65.0a*	*13.20*	*<0.0001*
Nitrogen (%)	Without	2.90b	2.46c	3.31a	2.90b	2.54c	2.44c	2.77b	*19.49*	*<0.0001*
	With	3.08	2.79	2.90	2.96	2.92	2.75	2.90	*0.83*	*0.56*
Potassium (%)	Without	1.76cd	2.06b	2.59a	1.68cd	1.92bc	1.28e	1.54d	*33.3*	*<0.0001*
	With	2.00c	2.19b	2.37a	1.64d	2.20ab	1.42e	1.56de	*41.2*	*<0.0001*

Mean values with different letter(s) in a row are significantly different at $P < 0.05$.

III.1.5.2. Biophysical characteristics that play a role in resistance

In the first selection (2011), there was no significant difference in leaf trichome density in leaves from middle (P = 0.65) and bottom (P = 0.29) strata among the accessions. However, in the younger leaves of VI033805, it was significantly higher than VI051114 which was among the most susceptible in the selected accessions (P = 0.04) (Table XIV). For leaf toughness, there was no significant difference for the force needed to puncture the leaves from top (P = 0.87), middle (P = 0.86) and bottom (P = 0.76) strata (Table XIV).

For the second (2012) and third (2013) selections, there were significant differences in trichome density in leaves from top (P < 0.0001), middle (P = 0.005) and bottom (P = 0.0004) strata among the accessions. In general, it was significantly higher in the top three moderately resistant ones (VI041210, VI057245 and VI033824) than in the other ones (Table XIV). It was also significantly lower in the susceptible VI060794. The force needed to puncture the leaves were similar from top (P = 0.41), middle (P = 0.11) and bottom (P = 0.14) strata (Table XIV).

Table XIV: trichome density (number of trichomes per cm^2) and leaf toughness (g) in Taiwan

Selection	Accession	Leaf trichome density			Leaf toughness		
		Bottom	Middle	Top	Bottom	Middle	Top
2011 Selection conducted in Taiwan in 2012	VI057245	4.94	10.32	33.52ab	22.32	22.22	22.02
	VI036213	6.98	15.12	46.62ab	21.02	21.36	20.92
	VI033805	4.66	13.54	59.04a	20.14	20.52	20.56
	VI051114	7.16	13.06	32.40b	19.92	20.42	20.46
	$F_{(3,16\ df)}$-Value	1.38	0.56	3.66	0.4	0.26	0.24
	P-Value	0.29	0.65	0.04	0.76	0.86	0.87
2012 and 2013 Selection conducted in Taiwan in 2013	VI060794	0.67b	1.42c	0.92d	7.28	10.88	12.18
	VI039614	1.33b	3.75abc	11.25bc	8.35	09.27	12.37
	VI041210	2.67ab	5.75ab	20.5a	7.28	11.85	13.53
	VI057245	2.67ab	6.67a	11.75bc	6.58	10.95	10.95
	VI060688	1.08b	1.92bc	6.83dc	10.6	14.05	13.78
	VI060818	1.58b	2.33bc	5.58dc	7.52	10.55	10.33
	VI033824	4.92a	4.83abc	15.25ab	9.8	11.04	13.35
	$F_{(6,14\ df)}$-Value	8.83	5.16	14.15	1.94	2.20	1.09
	P-Value	0.0004	0.005	<.0001	0.14	0.11	0.41

Mean values with different letters in a column are significantly different (P < 0.05).

The selected resistant accessions were evaluated together with the susceptible checks for chlorophyll, leaf trichomes and leaf toughness during the combined study. There were significant differences among accessions in leaf trichome density for all the strata and in leaf toughness for the top stratum only. There were no significant differences among accessions in chlorophyll for all the strata. The two most susceptible accessions (VI060794 and Kirikou) and Gombo caféier had significantly the lowest leaf trichome density than all other accessions (Table XV).

Table XV: studies on biophysical parameters between susceptible and resistant accessions conducted in Cameroon in 2014.

Accession	Chlorophyll (µg/g)			Trichome density (per cm^2)			Toughness (g)		
	Bottom	Middle	Top	Bottom	Middle	Top	Bottom	Middle	Top
Kirikou	2.5	3.2	2.7	1.08de	1.83de	3.08e	12.75	12.83	12.75bcd
Gombo caféier	2.5	3.1	2.7	1.92cde	2.00de	2.58e	15.58	18.83	16.58abc
VI033805	2.1	2.7	2.8	10.67a	21.4a	24.8ab	16.42	15.33	18.33a
VI033824	2.4	3.6	2.9	9.08ab	13.0bc	17.5abc	15.17	15.25	14.75abcd
VI036213	2.1	3.1	2.8	4.67cd	8.08bcde	20.4ab	13.58	13.67	17.08ab
VI039614	1.9	1.7	1.4	5.83bc	10.8bc	14.17bcd	15.50	14.83	16.33abc
VI041210	2.8	2.9	3.3	1.92cde	9.75bcd	23.58ab	14.92	14.67	14.92abcd
VI051114	2.5	3.3	2.9	3.17cd	15.42ab	26.17a	12.92	13.25	13.92abcd
VI057245	2.2	2.7	3.1	5.25bcd	5.75cde	9.3cde	12.33	13.50	11.17d
VI060688	2.9	3.9	3.4	2.50cde	5.92cde	6.5de	14.75	13.58	12.92bcd
VI060794	2.5	3.0	2.8	0.17e	0.17e	0.17e	11.42	14.75	11.92cd
VI060818	3.2	3.3	3.2	5.17bcd	7.17cde	22.08ab	13.92	13.92	15.75abcd
P-value	0.35	0.097	0.08	0.0001	0.0002	<.0001	0.395	0.456	0.034
$F_{11,24df}$-value	1.22	1.97	2.06	6.07	5.80	8.01	1.11	1.02	2.43

Mean values with different letter(s) in a column are significantly different at $P < 0.05$.

III.1.6. Assessing the mechanisms of resistance

III.1.6.1. Tolerance

❖ Okra production cycle during confirmatory screening

There were significant differences among accessions in days to anthesis, commercial maturity and duration of crop cycle (Table XVI). Gombo caféier had significantly higher duration in all parameters than most other one. VI060794 showed significantly higher duration only in crop growth cycle, but not significantly different from Gombo caféier (Table XVI).

Table XVI: flowering, fruiting and crop cycle duration during two seasons of the confirmatory screenings in 2014 in Yaoundé

	Anthesis (days)		Commercial maturity (days)		End of production (days)	
Accession	First season	Second season	First season	Second season	First season	Second season
Kiriko	42.0e	57.0b	51.0de	68.0cd	110.1bc	102.0abc
Gombo Caféier	88.7a	90.7a	108.7a	100.7a	137.7a	115.0a
VI033805	62.0c	60.7b	72.0c	87.0b	107.7bcd	95.0abc
VI033824	40.3e	56.3b	52.0de	68.0cd	105.7bcd	95.0abc
VI036213	57.7cd	61.3b	68.3c	71.3cd	109.7bc	98.0abc
VI039614	34.7e	54.0b	45.7e	62.7d	87.0e	79.0c
VI041210	75.0b	58.0b	96.3b	64.0d	105.3bcd	92.5abc
VI051114	54.0cd	68.0b	64.7c	71.0cd	94.7de	98.0abc
VI057245	52.3d	62.0b	61.0cd	80.0bc	113.7b	106.0ab
VI060688	40.0e	59.7b	48.0e	68.0cd	98.7cde	81.0c
VI060794	50.7d	60.3b	64.7c	69.7cd	137a	113.3a
VI060818	39.0e	58.7b	49.3e	72.5cd	99.7bcde	89.0bc
P-value	<.0001	0.002	<.0001	0.0002	<.0001	0.039
F-value	29.59	4.46	31.57	9.25	12.76	3.17
Trt./error df	11,24	11,20	11,24	11,13	11,24	11,10

Means with different letters in a column are significantly different at $P < 0.05$; Trt = treatment.

❖ Okra yield and plant parameters during confirmatory screening

There were significant differences in yield among the accessions. The farmers' variety Kirikou and VI060794 had the highest yield. Although there were significant differences in the yields for the second season ($P < 0.0001$), these yields were generally low, except for VI041210 that produced significantly higher number of pods per plant, than all other ones (Table XVII). There were significant differences among accessions during the two seasons in plant height ($P = < 0.0001$, $F = 6.75$; $P = 0.019$, $F = 2.77$) and stem diameter ($P = < 0.0001$, $F = 7.36$; $P = 0.003$, $F = 3.87$) and during the first season only, in pod length ($P = 0.01$, $F = 2.92$) and leaf area ($P = 0.008$, $F = 3.2$) (Table XVII). Gombo caféier had the highest plant height, stem diameter, and leaf area in the first season while VI051210 had the highest value for the same three parameters in the second season. VI060794 had the highest stem diameter during the second season and highest pod width during the two seasons, but not significantly different from VI051210 during second season, Gombo caféier and Kirikou during all seasons. VI051114 and VI060688 had the highest pod length but not significantly different from most accessions. (Table XVII).

Table XVII: plant parameters during two seasons of the confirmatory screening in 2014 at Nkolbisson in Yaoundé

	Yield (Pods/plant)		Plant height (cm)		Stem diameter (cm)		Pod length (cm)		Pod width (cm)		Leaf area (cm^2)		Pod size (g/pod)	
Accession	First	Second	First	Second	First	Second	First	Second	First	Second	First	Second	First	Second
Kirikou	23.7a	1.5b	91bcd	43.2ab	1.6cd	1.0abcd	5.6bc	4.5a	1.9ab	2.4ab	222abc	108.6abc	17.5ab	9.4abc
Gombo Caféier	11.5b	1.8b	142a	35.6bcd	2.8a	1.3ab	4.5c	4.5a	2.2a	2.6a	282a	110.8abc	13.2ab	6.7bcd
VI033805	4.1bc	0.2b	79cde	32.0bcd	2.1bc	1.1abc	7.6ab	6.6a	1.5cd	2.0bcde	256ab	109.2abc	17.9ab	6.7bcd
VI033824	7.2 bc	1.9b	61de	17.9cd	1.5d	0.6cd	8.9a	7.2a	1.4cde	1.7def	179bc	70.1bc	18.6a	6.5bcd
VI036213	4.2bc	0.9b	50e	29.9bcd	1.8cd	1.2abc	7.7ab	5.2a	1.1de	1.6ef	185bc	116.8abc	13.1ab	5.6bcd
VI039614	9.6b	1.0b	81cde	27.7bcd	1.4d	0.6cd	7.9ab	5.5a	1.1de	1.5f	139c	49.0c	10.4ab	3.8cd
VI041210	1.6c	7.7a	49e	61.3a	2.4b	1.6a	7.2abc	7.3a	1.0e	2.3abc	254ab	266.7a	9.7b	9.4abc
VI051114	4.0bc	0.5b	52e	25.7bcd	1.5d	0.7cd	9.4a	8.6a	1.2de	1.9cdef	161c	62.6c	18.8a	14.0a
VI057245	6.6bc	0.2b	66de	16.3d	1.7cd	0.5d	7.8ab	3.4a	1.7bc	1.6def	142c	30.4c	14.6ab	3.5d
VI060688	7.8bc	0.6b	105bc	44.0ab	1.7cd	0.7cd	9.2a	5.3a	1.4cde	2.0bcd	199abc	59.1c	15.8ab	5.8bcd
VI060794	20.5a	2.1b	98bcd	35.3bcd	1.5d	1.4a	4.9bc	6.7a	2.3a	2.5a	157c	232.0ab	14.4ab	6.9bcd
VI060818	10.7b	0.9b	120ab	41.2abc	1.6cd	0.8bcd	7.2abc	6.7a	1.7bc	2.4ab	196abc	65.5c	17.8ab	9.8ab
P-value	<.0001	<.0001	<.0001	0.019	<.0001	0.003	0.01	0.69	<.0001	<.0001	0.008	0.096	0.226	0.055
F-value	8.74	6.59	6.75	2.77	7.36	3.87	2.92	0.74	10.23	9.53	3.2	1.88	1.43	11,16
Trt and error df	11,24	11,23	11,24	11,23	11,24	11,23	11,24	11,15	11,24	11,15	11,24	11,23	11,24	2.39

Mean values with different letters in a column are significantly different at $P < 0.05$; Trt = Treatment, df = Degree of freedom.

III.1.6.2. Antixenosis

❖ Choice and no-choice test

Studies on settling behaviour showed that aphids did not discriminate, under choice conditions, between susceptible and resistant okra accessions 72 h and 48 h after release, for the first selection and experiment with all selections respectively. There were no significant difference among the accessions in non-preference (P = 0.241 and P = 0.972 respectively) (Table XVIII). In addition, during the second and third selections and in experiment with all selections, *Aphis gossypii* resistance was shown for aphid permanence on the infested leaf after 72 h of release under no choice condition (antixenosis). Although the percent non-preference was higher in some resistant and some susceptible accessions, there were no significant differences among the resistant accessions in terms of aphid permanence on infested leaves for second and third selection (P = 0.827) and for experiment with all selections (P = 0.853) (Table XVIII).

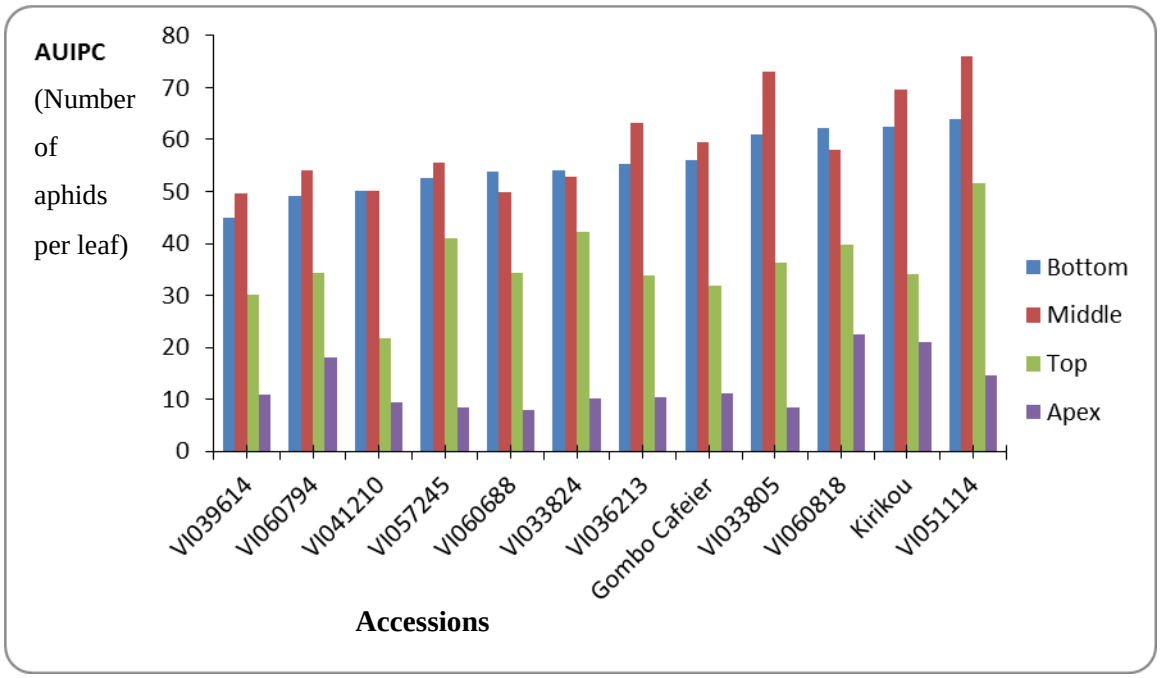
Table XVIII: settling behaviour of aphids on okra with the first and second selections conducted in Cameroon with two Cameroonian varieties

Accessions	1st selection tested in Taiwan in 2012 (choice test)	All selections evaluated in 2015 (Choice test)	2nd and 3rd selection tested in Taiwan in 2013 (no-choice)		All selection evaluated in 2015 (no-choice)
	Number of aphids per leaf after 72 h	Number of aphids per leaf after 48 h	Number of aphids per leaf after 72 h	Plants with < 5 adults per infested leaf after 72 h (%)	Plants with < 5 adults per infested leaf after 72 h (%)
VI051114	15.0	4.4			22.2
VI036213	17.00	4.2			44.4
VI033805	15.83	3.7			55.6
VI057245	26.67	5.6	8.7	11.11	22.2
VI060794		3.7	8.1	22.22	55.6
VI039614		3.1	7.8	11.11	55.6
VI041210		4.0	10.3	11.11	33.3
VI033824		4.5	8.1	0.00	33.3
VI060688		3.3	8.3	11.11	22.2
VI060818		2.4	7.6	11.11	33.3
Gombo caféier		3.3			33.3
Kirikou		3.2			33.3
Pr>Chi-Square	0.2407	0.972	0.6861	*0.827*	0.853
χ^2 =	7.815	3.944	3.931	2.857	6.300

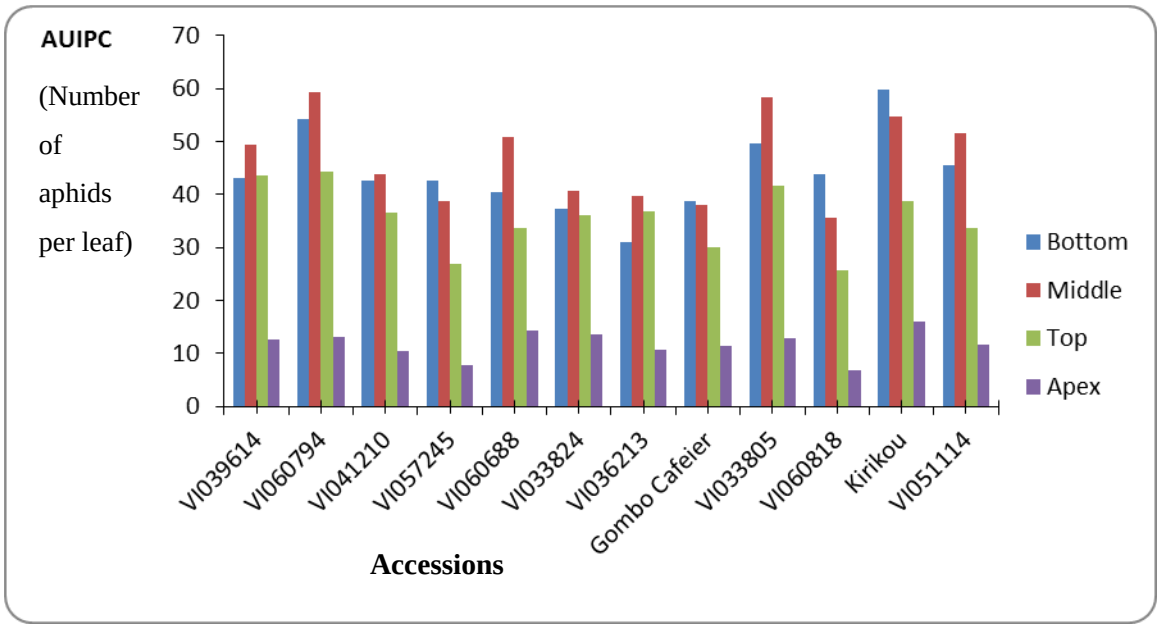
"Blank space indicates that the variety was not tested.

❖ Aphid spatial distribution during confirmatory screening

The spatial distribution of aphids on okra indicated that aphid infestation is highest on the middle leaves, higher on the bottom stratum, high for the top stratum and low for the terminal bud during both seasons (Figure 47 a and b).



(a)



(b)

Figure 47: spatial distribution of *Aphis gossypii* on okra accessions in 2014 at Nkolbisson, Yaoundé; a: first season and b: second season.

III.1.6.3. Antibiosis

- Constitutive analysis of antibiosis

○ Development of *Aphis gossypii* at vegetative (2 WAT) and reproductive (10 WAT) stage of plants previously uninfested with aphids

At vegetative stage of the plants previously not infested with aphids, there were no significant difference in all development parameters among accessions of the first and second selections, except in numph period (P = 0.015). VI033805, one of the most susceptible accessions, had the lowest nymph period. Gombo caféier, the most resistant had highest nymphal mortality.

In the third selection, there were significant difference among accessions in the number of moults per aphid (P = 0.02) and generation time (T_o) (P = 0.024). The number of moult was the lowest for VI057245, one of the most resistant accessions, but duration of moulting was the highest in this accession (VI057245) and lowest in the susceptible Kirikou (Table XIX).

Table XIX: development of *Aphis gossypii* at vegetative stage of plants previously not infested

Selection	Accession	Nymph Mortality rate (%)	Mean number of moults/ aphid	Instar duration (days)					Nymph Period (days)	Mean duration of each moult (days)	T_o (days)
				1st	2nd	3rd	4th	5th			
1st and 2nd selections	VI033824	0.0	3.2	3.0	2.0	1.0	0.3	0.2	6.5ab	2.1	9.9
	VI051114	0.0	3.9	1.8	1.8	1.4	0.9	0.2	6.1bc	1.6	7.5
	VI036213	0.0	3.6	2.4	1.3	1.6	0.4	0.2	6.3ab	1.8	3.0
	VI033805	0.0	3.5	2.0	1.7	1.5	0.5	0.0	5.7c	1.6	7.6
	Kirikou	0.0	4.0	1.8	1.5	2.0	0.7	0.8	6.8a	1.8	7.8
	Gombo Caféier	11.1	3.8	1.3	3.0	1.2	0.7	0.2	6.3ab	1.7	6.3
	$F_{5,12}$-value	1.00	0.66	2.27	1.74	0.7	0.61	1.75	4.56	1.16	2.59
	P-value	0.46	0.66	0.11	0.2	0.63	0.69	0.2	0.015	0.38	0.08
3rd selection	VI039614	11.1	3.2ab	3.7	3.2	1.3	0.2	0.0	8.8	2.8abc	9.9ab
	VI041210	41.7	4.0ab	2.2	2.7	2.1	0.8	0.2	8.1	2.0bcd	7.8b
	VI057245	19.4	2.7b	4.3	2.2	1.7	0.3	0.0	8.8	3.2a	15.9a
	VI060688	0.00	3.0ab	4.0	2.7	1.3	0.3	0.0	8.3	3.0ab	9.3ab
	VI060818	16.7	4.3a	1.5	1.8	2.0	1.3	0.3	7.0	1.6d	9.6ab
	Gombo caféier	0.00	3.4ab	3.3	2.6	1.1	0.6	0.3	8.0	2.4abcd	8.3b
	Kirikou	28.3	3.9ab	1.7	2.2	1.9	0.8	0.2	6.8	1.8cd	6.7b
	VI060794	11.1	3.3ab	3.5	3.1	1.7	0.4	0.2	8.8	2.7abcd	9.0ab
	$F_{7,16}$-value	1.68	3.38	2.63	0.42	0.32	2.73	0.60	0.75	2.96	3.27
	P-value	0.18	0.02	0.05	0.87	0.94	0.05	0.75	0.63	0.03	0.02

To = generation time.

Mean values with different letter(s) in a column are significantly different at P < 0.05.

At reproductive stage, there were no significant differences among the accessions of the first and second selections in all developmental attributes (Table XX).

In the third selection, there were significant differences among accessions in number of moults (P = 0.003), fourth instar development (P = 0.0003) and duration per moult (P = 0.0005). Mortality was higher on Gombo caféier while number of moults was the lowest in VI041210, which is among the most resistant accessions. The duration of each moult was lower on VI060818 followed by Kirikou, one of the susceptible checks (Table XX).

Table XX: development of *Aphis gossypii* at reproductive stage of plants previously not infested

Selection	Accession	Nymph mortality rate (%)	Number of moults/ aphid	Instar duration (days)					Nymph period (days)	Duration of each moult (days)	T_o (days)
				1st	2nd	3rd	4th	5th			
1st and 2nd	VI033824	11.1	2.3	3.7	2.4	0.6	0.0	0.0	6.7	3.0	7.4
	VI051114	08.3	2.3	3.3	1.4	0.8	0.4	0.0	6.0	2.7	8.4
	VI036213	0.00	2.9	3.3	1.6	0.7	0.6	0.4	6.5	2.5	11.5
	VI033805	16.7	3.0	2.0	2.2	1.5	0.7	0.0	6.3	2.2	6.9
	Kirikou	16.7	3.0	2.7	2.7	0.7	0.7	0.0	6.7	2.4	8.4
	Gombo Caféier	0.00	2.7	3.0	1.7	2.0	2.7	0.0	9.3	4.1	7.3
	$F_{5,12}$-value	0.46	0.3	0.4	0.57	0.89	0.66	1.0	1.4	1.1	0.93
	P-value	0.8	0.9	0.84	0.7	0.52	0.66	0.46	0.29	0.41	0.50
3rd	VI039614	0.0	3.3ab	2.7	2.5	1.5	0.7acd	0.5	7.8	2.4abc	5.9
	Kirikou	0.0	3.9a	2.6	1.8	1.1	1.5a	0.6	7.6	1.9bc	5.6
	VI041210	0.0	2.4b	2.5	2.6	0.5	0.1d	0.0	5.6	2.4abc	6.4
	VI057245	0.0	3.5ab	3.0	1.6	1.1	0.8abcd	0.7	7.2	2.0bc	6.4
	VI060688	0.0	3.8a	2.7	1.9	1.4	1.3ab	0.2	7.4	2.0bc	7.8
	VI060818	5.6	4.1a	1.9	2.2	1.8	1.0abc	0.5	7.2	1.8c	8.7
	Gombo Caféier	6.7	3.0ab	3.6	2.4	1.0	0.4cd	0.0	7.4	2.5ab	9.5
	VI060794	0.0	2.8ab	3.3	3.0	1.2	0.3cd	0.0	7.8	2.8a	6.6
	$F_{7,16}$-value	0.86	5.08	0.96	0.85	1.79	7.93	1.12	2.08	7.28	1.86
	P-value	0.56	0.003	0.49	0.56	0.16	0.0003	0.40	0.11	0.0005	0.14

To = generation time.

Mean values with different letter(s) in a column are significantly different at P < 0.05.

- Reproductive performance of *Aphis gossypii* during vegetative (2 WAT) and reproductive (10 WAT) stage of plants previously uninfested with aphids

In the first and second selections, all reproductive attributes but one, net reproduction rate, showed significant differences among accessions (Table XXI). VI033824 had the highest

reproductive time (P = 0.021), population doubling time (P = 0.046) and lower finite rate of increase (P = 0.038) than with VI036213; also the highest biological cycle than with VI033805 and VI036213 (P = 0.013). VI036213 also had significantly higher intrinsic rate of increase than all accessions, except with VI051114 (P = 0.019). VI033824 and the susceptible check Kirikou had the highest biological cycle duration (Table XXI).

The third selection results revealed significant differences among accessions in intrinsic rate of increase (P = 0.014), finite rate of increase (P = 0.019) and population doubling time (DT) (P=0.009) (Table XXI). Susceptible Kirikou had the highest intrinsic rate of increase and finite rate of increase, which was significantly higher than with resistant VI057245. The population doubling time with VI057245 was significantly higher than with VI041210, Kirikou and Gombo caféier (Table XXI).

Table XXI: reproductive performance of *Aphis gossypii* at vegetative stage of plants previously not infested with aphids

Selection	Accession	Reproductive time (RT)(days)	No. of aphids/ individual (R_o)	No. of aphids /individual/ day(r)	Finite rate of increase (λ)	Population doubling time (days)	Biological cycle (days)
1st and 2nd selection	VI033824	20.0a	78.6	0.4b	1.5b	1.6a	26.5a
	VI051114	16.3ab	55.4	0.6ab	1.8ab	1.3ab	22.4ab
	VI036213	5.9b	21.8	1.1a	3.0a	0.7b	12.2c
	VI033805	13.3ab	40.8	0.5b	1.7ab	1.4ab	19.0bc
	Kirikou	15.7ab	50.6	0.5b	1.7ab	1.4ab	22.5ab
	Gombo Caféier	12.3ab	43.4	0.6ab	1.9ab	1.2ab	18.7ac
	$F_{5,12}$-value	4.13	1.38	4.19	3.4	3.20	4.67
	P-value	0.021	0.30	0.019	0.038	0.046	0.013
3rd selection	VI060794	18.2	37.3	0.36ab	1.4ab	2.0ab	27.0
	VI039614	20.3	34.0	0.34ab	1.4ab	2.1ab	29.2
	VI041210	16.7	40.4	0.47ab	1.6ab	1.5b	24.8
	VI057245	25.4	44.0	0.25b	1.3b	2.9a	34.3
	VI060688	16.8	25.9	0.37ab	1.5ab	2.0ab	25.2
	VI060818	23.0	48.7	0.39ab	1.5ab	1.7ab	30.0
	Kirikou	12.3	36.1	0.54a	1.7a	1.3b	10.1
	Gombo Caféier	19.0	40.0	0.46ab	1.6ab	1.6b	26.9
	$F_{5,12}$-value	1.54	0.43	3.72	3.46	4.12	1.71
	P-value	0.23	0.87	0.014	0.019	0.009	0.18

Mean values with different letter(s) in a column are significantly different at P < 0.05.

During the reproductive stage of the plants, there were no significant differences among the accessions for the first and second selections in any parameter.

The third selection showed significant differences among accessions in net reproduction rate (P = 0.007), intrinsic rate of increase (P = 0.04) and finite rate of increase (0.041) (Table XXII). Gombo caféier had the highest net reproduction rate while VI060794 had the highest intrinsic rate of increase and finite rate of increase (Table XXII).

Table XXII: reproductive performance of *Aphis gossypii* on selected accessions at reproductive stage of plants previously not infested with aphids

Selection	Accession	Reproductive time (RT) (days)	No. of aphids/ Individual (R_o)	No. of aphids/ individual/day(r)	Finite rate of increase (λ)	Population doubling time (DT) (days)	Biological cycle (days)
1st and 2nd selection	VI033824	15.1	44.0	0.5	1.7	1.4	21.8
	VI051114	17.1	43.5	0.5	1.6	1.6	23.1
	VI036213	21.7	47.4	0.4	1.4	2.1	28.2
	VI033805	13.7	49.9	0.6	1.8	1.3	20.0
	Kiriko	15.5	36.0	0.4	1.5	1.6	22.8
	Gombo Caféier	13.5	16.7	0.4	1.5	2.0	22.8
	$F_{5,12}$-value	1.22	2.34	2.0	2.03	1.52	1.33
	P-value	0.36	0.11	0.15	0.15	0.26	0.32
3rd Selection	VI060794	12.6	38.7ab	0.6a	1.8a	1.3	20.4
	VI039614	11.4	23.4b	0.5a	1.7ab	1.4	19.2
	VI041210	14.8	31.7ab	0.5a	1.7ab	1.3	20.4
	VI057245	13.7	22.0b	0.5ab	1.6abc	1.5	20.9
	VI060688	15.7	21.0b	0.4ab	1.5bc	1.8	23.0
	Kirikou	12.7	20.1b	0.5a	1.7ab	1.3	20.3
	Gombo caféier	20.7	56.9a	0.4ab	1.5abc	1.7	28.0
	VI060818	17.4	22.5b	0.35b	1.4c	2.1	24.6
	$F_{5,12}$-value	1.77	4.3	2.83	2.81	2.32	1.71
	P-value	0.16	0.007	0.040	0.041	0.077	0.18

Mean values with different letter(s) in a column are significantly different at P < 0.05.

- Analysis of antibiosis by induction

○ Development of *Aphis gossypii* during vegetative (2 WAT) and reproductive (10 WAT) stage of plants previously infested with aphids

For plants previously infested with aphids, there were significant differences among accessions in duration of fourth instar (P = 0.007), nymph period (P = 0.0002) and in mortality of nymphs. Mortality was lowest in the most susceptible Kirikou and VI033805. The developmental time or nymph period was the highest on VI041210 (Table XXIII).

Table XXIII: development of *Aphis gossypii* on selected accessions at vegetative stage of plants previously infested with aphids

Accession	Nymph Mortality rate (%)	Number of moults/ aphid	Instar duration (days)					Nymph period (days)	Duration of each moult (days)	T_o (days)
			1st	2nd	3rd	4th	5th			
VI033824	0.0c	2.6	3.0	2.4	1.3	0.4b	0.2	7.3b	3.1	13.6
VI051114	8.3bc	2.8	4.1	2.3	1.4	0.3b	0.8	7.9b	2.8	13.0
VI036213	18.9abc	3.5	3.4	1.8	1.8	1.0ab	0.2	8.1b	2.4	8.7
VI033805	0.0c	3.2	2.8	2.3	0.5	0.5b	0.2	6.3b	2.2	9.9
Kiriko	0.0c	3.7	1.4	3.1	2.9	0.5b	1.5	8.9b	2.5	11.1
Gombo Caféier	6.7c	3.5	3.5	2.5	1.7	0.5b	0.3	8.4b	2.5	10.3
VI060794	36.1a	3.1	4.8	2.1	1.0	0.7b	0.7	9.2b	4.4	9.8
VI039614	16.7abc	2.8	3.3	3.0	1.5	0.3b	0.0	8.2b	3.1	6.8
VI041210	11.1abc	2.8	5.8	5.3	2.7	0.3b	0.0	14.2a	5.1	8.2
VI057245	5.6c	2.7	2.6	2.6	1.1	0.2b	0.0	6.5b	2.4	9.2
VI060688	11.1abc	4.0	3.0	2.8	2.7	2.2a	0.0	10.7ab	2.7	7.3
VI060818	33.3ab	3.7	3.0	3.2	1.0	0.8ab	0.2	8.5b	2.5	8.7
$F_{11,24}$-value	2.44	0.84	0.68	1.53	1.22	3.33	0.78	5.55	1.15	1.24
P-value	0.033	0.60	0.74	0.19	0.33	0.007	0.65	0.0002	0.368	0.32

To = generation time.

Mean values with different letter(s) in a column are significantly different at P < 0.05.

During the reproductive stage of the plant, significant differences were found among accessions in duration of first instar nymph (P = 0.036) and in generation time (To) (P = 0.0086). The duration of first instar nymph was significantly higher with VI033805 than with VI051114. The generation time was significantly higher on VI033805 and lower on VI060818 and VI033824

(Table XXIV). Although there was no significant difference among accessions in nymphal mortality, one of the susceptible accessions, VI060794 had 0 % mortality while one of the resistant accessions VI041210 had the highest percent nymphal mortality (Table XXIV).

Table XXIV: development of *Aphis gossypii* on selected accessions at reproductive stage of plants previously infested with aphids

Accession	Nymph Mortality rate (%)	Mean number of moults/ aphid	Instar duration (days)					Nymph period (days)	Duration of each moult (days)	To (days)
			1st	2nd	3rd	4th	5th			
VI033824	11.1	2.3	3.8ab	2.5	1.2	0.2	0.0	7.7	3.5	5.2b
VI051114	8.3	3.0	1.4b	2.4	2.7	0.4	0.0	7.0	2.4	8.3ab
VI036213	13.3	2.9	2.4ab	2.3	0.7	0.6	0.0	5.9	2.1	9.1ab
VI033805	4.8	2.8	4.7a	2.1	0.8	0.6	0.3	8.4	3.1	14.7a
Kirikou	11.1	2.9	2.9ab	2.5	1.3	0.7	0.2	7.6	3.1	11.4ab
Gombo Caféier	6.7	2.8	3.1ab	2.5	1.4	0.2	0.0	7.1	2.6	11.2ab
VI060794	0.0	3.2	1.9ab	1.9	2.1	0.6	0.0	6.4	2.0	11.7ab
VI039614	0.0	2.6	3.2ab	3.4	1.4	0.2	0.0	8.2	3.3	7.4ab
VI041210	27.8	1.8	3.8ab	0.7	1.5	0.0	0.0	6.0	3.6	10.7ab
VI057245	0.0	2.1	4.3ab	1.8	0.7	0.0	0.0	6.8	3.4	8.8ab
VI060688	0.0	2.5	3.0ab	2.3	1.2	0.3	0.0	6.8	2.7	7.4ab
VI060818	11.1	3.3	2.9ab	1.3	1.7	0.7	0.0	6.7	2.1	5.8b
$F_{11,24}$-value	1.02	0.98	2.39	1.07	0.58	0.94	0.92	1.59	1.53	3.18
P-value	0.46	0.49	0.036	0.42	0.83	0.52	0.54	0.17	0.19	0.009

T0 = generation time.

Mean values with different letter(s) in a column are significantly different at $P < 0.05$.

- Reproductive performance during vegetative (2 WAT) and reproductive (10 WAT) stage of plants previously infested with aphids

During the vegetative stage of the plant previously infested with aphids, there were no significant differences among accessions in any reproduction parameters of *Aphis gossypii* (Table XXV).

Table XXV: reproductive performance of *Aphis gossypii* on selected accessions at vegetative stage of plants previously infested with aphids

Accession	Reproductive time (RT) (days)	No. of aphids/ individual (R_o)	No. of aphids/ individual /day(r)	Finite rate of increase (λ)	Population doubling time (DT) (days)	Biological cycle (days)
VI033824	22.2	49.3	0.3	1.3	2.4	29.5
VI051114	24.1	51.8	0.3	1.4	2.3	32.0
VI036213	17.9	43.5	0.4	1.5	1.6	26.0
VI033805	19.3	47.3	0.4	1.5	1.9	25.7
Kirikou	23.3	44.1	0.3	1.4	2.1	32.3
Gombo Caféier	17.6	59.2	0.4	1.5	1.8	25.9
VI060794	16.7	46.6	0.4	1.5	1.9	25.9
VI039614	14.2	24.7	0.4	1.6	1.6	22.3
VI041210	17.0	27.1	0.4	1.5	1.8	31.2
VI057245	19.1	50.7	0.4	1.5	1.6	25.5
VI060688	14.2	20.4	0.4	1.5	1.7	24.8
VI060818	19.3	36.2	0.4	1.6	1.7	27.8
$F_{11,24}$-value	0.90	1.16	0.84	0.79	1.03	0.76
P-value	0.552	0.366	0.607	0.65	0.451	0.673

At the reproductive stage of plants previously infested with aphids, all parameters showed significant differences among accessions (Table XXVI). VI033805 had a reproductive time significantly higher than for VI033824, VI060688 and VI060818. Similar trend was observed in the biological cycle in addition to VI036213. VI033805 also had the net reproduction rate significantly higher than that of six of the accession (Table XXVI). Significantly higher intrinsic rate of increase, finite rate of increase and significantly lower population doubling time were observed with VI033824 than with VI033805, Kirikou, Gombo Caféier, VI060794 and VI041210 (Table XXVI).

Table XXVI: reproductive performance of *Aphis gossypii* on selected accessions at reproductive stage of plants previously infested with aphids

Accession	Reproductive time (RT) (days)	No. of aphids/ individual (R_o)	No. of aphids/ individual/ day(r)	Finite rate of increase (λ)	Population doubling time (DT) (days)	Biological cycle (days)
VI033824	11.0b	28.1b	0.6a	1.9a	1.1b	18.7b
VI051114	15.6ab	30.8b	0.4ab	1.6ab	1.7ab	22.6ab
VI036213	14.2ab	47.3ab	0.4ab	1.5ab	1.7ab	20.1b
VI033805	26.1a	82.1a	0.3b	1.4b	2.3a	34.5a
Kirikou	16.8ab	33.5ab	0.3b	1.4b	2.3a	23.3ab
Gombo Caféier	17.7ab	44.3ab	0.3b	1.4b	2.1ab	24.7ab
VI060794	19.3ab	43.8ab	0.3b	1.4b	2.2a	25.8ab
VI039614	13.4ab	20.5b	0.4ab	1.5ab	1.7ab	21.6ab
VI041210	16.5ab	27.8b	0.3b	1.4b	2.3a	22.5ab
VI057245	16.6ab	45.1ab	0.4ab	1.6ab	1.6ab	23.4ab
VI060688	11.0b	19.7b	0.4ab	1.5ab	1.8ab	17.9b
VI060818	10.2b	14.2b	0.6ab	1.6ab	1.6ab	16.8b
$F_{11,24}$-Value	2.75	3.35	3.82	3.83	3.49	3.25
P-value	0.018	0.0065	0.0029	0.0029	0.0051	0.008

Mean values with different letter(s) in a column are significantly different at $P < 0.05$

- Aphid population and seasonal dynamics during confirmatory screening

Figure 48 shows that aphid populations change differently with time amongst the accession with no clear peak infestation period. However, there was generally a slight decreased in infestation in May and a rise in April and June. In the beginning of June, the aphid population on one of the resistant accession (VI041210) decreased (Figure 48) while the other resistant accession VI057245 started with low infestation which increased steadily and attained an intermediate level. The third resistant variety Gombo caféier maintained an intermediate level of infestation throughout the season. For the two most susceptible varieties, although VI060794 started as the least infested to the most infested at the end. The other susceptible variety Kirikou was the most infested at the begining and also one of the most infested at the end (Figure 48). Thus, there was limited fluctuation in infestation on the resistant varieties as opposed to susceptible ones.

In the second season, aphid population dynamics followed a similar trend in all accessions, with peak infestation in late October and early December and a drop in November (Figure 49). Similar to the first season, the two susceptible varieties showed greater fluctuation in

infestation than two of the resistant ones. The other (VI041210) like in the first season showed more fluctuation that was however, more pronounced in second season. The two most susceptible accessions (VI060794 and local Kirikou) maintained higher aphid populations while resistant ones maintained lower population throughout the season (Figure 49).

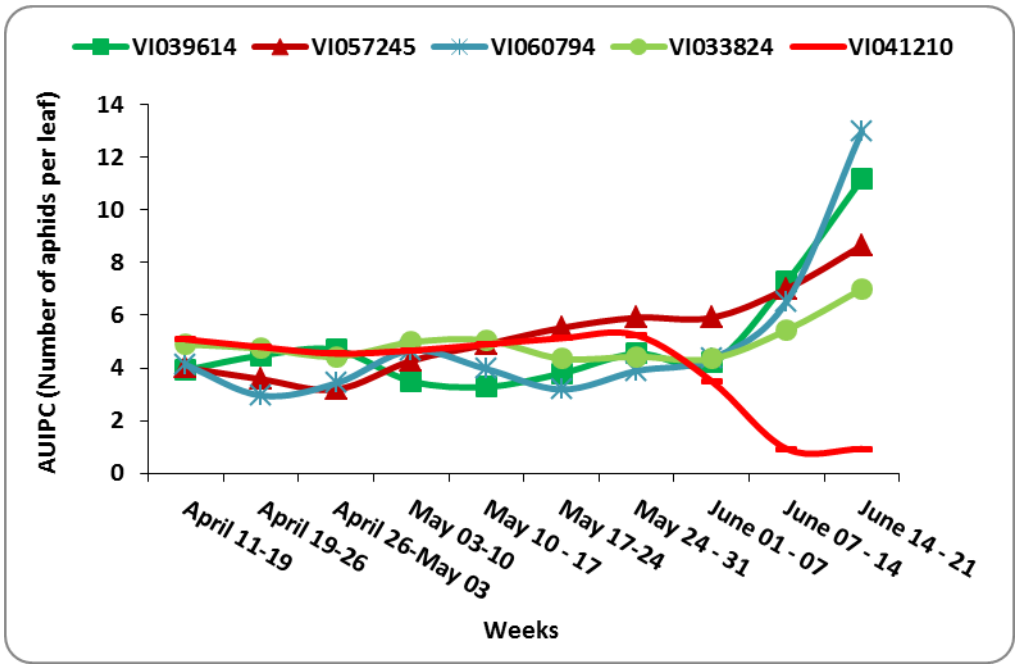


Figure 48: infestation pressure curve of aphids on the three most resistant and the two most susceptible varieties (first season of 2014) in Cameroon; red = susceptible ones.

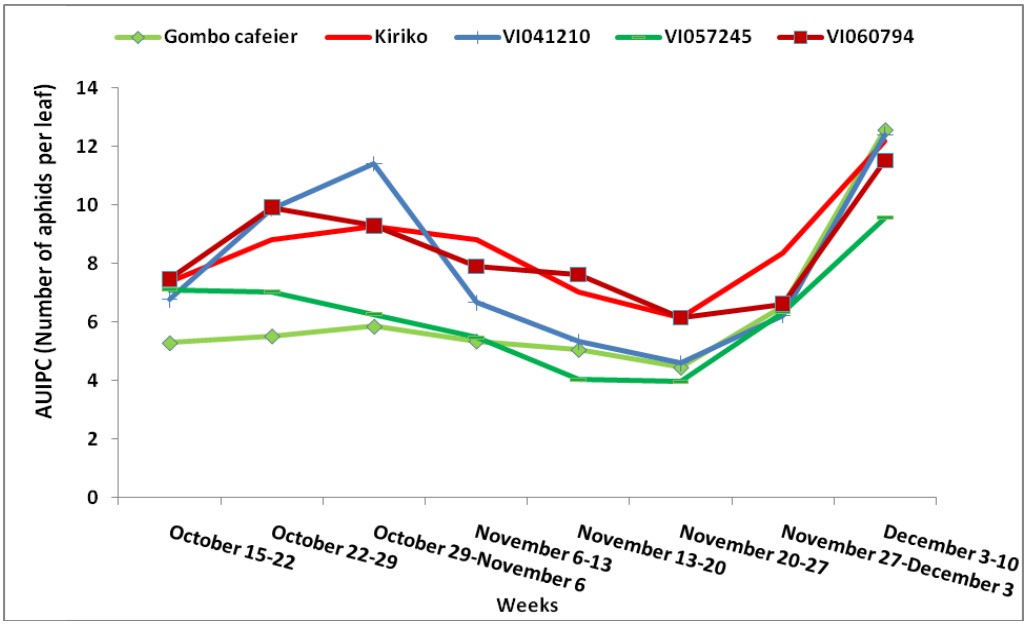


Figure 49: infestation pressure curve of aphids on the three most resistant and the two most susceptible varieties (second season of 2014) in Cameroon; red = susceptible ones.

III.1.7. Aphid resistance and yield performance of okra accessions under various agro-ecological climates in Cameroon

III.1.7.1. Effect of agro ecology on aphid infestation on different okra accessions

Results from the multi-location screening trials indicated that the varieties used by farmers in Cameroon were more susceptible to aphids than most of the selected resistant or moderately resistant accessions, across all agro-ecological zones. However, the common commercial variety “Gombo caféier” showed some resistance to *Aphis gossypii* in Evodoula in all seasons, in Buea during the second season, and in Maroua (Table XXVII). The resistance was regular with VI036213 in Foumbot, VI039614 in Buea, and VI051114 in Evodoula. In all the locations, the resistance of VI060794 was consistent, except during the first season at Foumbot and in Maroua. The trial in Maroua, which is a semi-arid region, was conducted only once from September to December 2014. In this trial, aphid did not infest two accessions (VI041210 and VI060818). The farmer variety from this region (Bascko Djo) was rated susceptible compared to the selected accessions, except VI060794, confirming the importance of aphids as a major pest especially on the local farmers’ varieties (Table XXVII). The resistance in the selected accessions also varied with time and space.

Aphid infestations were higher in the second season (60.4 to 114.6 aphids) than in the first (5.3 to 30.0 aphids) in Evodoula, Foumbot (53.3 to 76.4 aphids and 12.9 to 17.1 aphids respectively), but not in Buea (53.7 to 74.7 aphids and 48.1 to 75.8 aphids respectively). Nevertheless, seasons did not have an effect on resistance since the resistance change positively in some accessions and negatively in others, but had an effect on infestation since it was higher in the second (short) season except in Buea.

Table XXVII: Average number of aphids per leaf in different zones during the first and the second seasons at four locations in 2014

Accession	Buea		Evodoula		Foumbot		Maroua
	First	Second	First	Second	First	Second	Second
Gombo paysan	75.8 *Susceptible*	*68.9* Moderately Suscept					
Kirikou Bangourain			*26.5* Moderately Suscept	83.9 Moderately Suscept			
Bascko Djo Gombo					16.0 Moderately Suscept	65.0 Moderately Resist	5.8 *Susceptible*
caféier	63.7 Moderately Suscept	61.1 Moderately Resist	20.6 Moderately Resist	67.2 Moderately Resist	16.0 Moderately Suscept	70.4 Moderately Suscept	0.0 Resistant
VI033805	66.8 Moderately Suscept	57.5 Moderately Resist	26.4 Moderately Suscept	114.6 Highly Suscept	16.7 Moderately Suscept	75.3 *Susceptible*	1.4 Moderately Resist
VI033824	64.6 Moderately Suscept	53.7 Resistant	24.6 Moderately Suscept	80.6 Moderately Resist	15.5 Moderately Suscept	76.4 *Susceptible*	7.7 *Susceptible*
VI036213	55.0 Moderately Resist	74.7 *Susceptible*	29.5 Moderately Suscept	64.8 Resistant	10.3 Highly Resist	53.3 Resistant	3.5 Moderately Suscept
VI039614	52.6 Resistant	54.3 Resistant	08.6 Resistant	84.0 Moderately Suscept	17.1 Moderately Suscept	74.5 Susceptible	0.7 Moderately Resist
VI041210	63.6 Moderately Suscept	72.3 Susceptible	30.0 Moderately Suscept	78.1 Moderately Resist	15.6 Moderately Suscept	62.3 Moderately Resist	0.0 Resistant
VI051114	48.1 Resistant	64.2 Moderately Suscept	21.0 Moderately Resist	72.5 Moderately Resist	17.0 Moderately Suscept	68.3 Moderately Suscept	4.5 Moderately Suscept
VI057245	61.6 Moderately Suscept	65.7 Moderately Suscept	18.4 Moderately Resist	74.4 Moderately Resist	15.0 Moderately Resist	65.6 Moderately Resist	2.9 Moderately Resist
VI060688	66.8 Moderately Suscept	54.4 Moderately Resist	33.4 *Susceptible*	106.7 Susceptible	12.9 Resistant	71.5 Moderately Suscept	3.1 Moderately Suscept
VI060794	49.6 Resistant	56.6 Moderately Resist	16.8 Moderately Resist	60.4 Resistant	15.7 Moderately Suscept	55.1 Resistant	6.0 *Susceptible*
VI060818	60.4 Moderately Resist	57.6 Moderately Resist	05.3 Resistant	83.8 Moderately Suscept	16.3 Moderately Suscept	63.3Moderately Resist	0.0 Resistant
Onerall mean	60.7	61.8	21.8	80.9	15.4	66.8	3.0
Standard deviation (SD)	8.1	7.3	8.5	16.0	2.0	7.5	2.6

Resist. = Resistance

Suscept. = Susceptible

“Blank space indicates that the variety was not tested in corresponding location and season.

III.1.7.2. Yield performance of selected accession under different agroecologie

- Days to commercial maturity

Significant differences among accessions, in days to commercial maturity, were observed only in Evodoula in both seasons; Gombo caféier had the highest duration of commercial maturity. However, the duration was generally lower in the second season than in the first one except in Buea (Table XXVIII).

Table XXVIII: average days to commercial maturity during first and second seasons 2014 in different accessions at four locations

Accessions	Buea		Evodoula		Foumbot		Maroua
	First	Second	First	Second	First	Second	Second
Gombo Paysan	72.0	74.7					
Bangourain					88.0	71.7	
Kiriko			66.3c	55.0c			
Bascko Djo							58.3
Gombo Caféier	-	78.5	125a	74.5a	83.0	74.0	-
VI033805	-	80.0	88.7bc	71.0ab	96.0	67.0	78.5
VI033824	74.7	80.0	63.3c	61.3abc	72.0	64.7	64.0
VI036213	-	--	70.3c	64.3abc	119	74.0	63.7
VI039614	76.0	70.0	63.3c	53.7c	66.0	64.5	59.0
VI041210	-	70.0	87.5bc	64.3abc	93.3	--	66.0
VI051114	72.0	77.0	70.0c	67.0abc	120	74.0	72.0
VI057245	68.5	78.5	68.0c	62.0abc	120	72.7	61.3
VI060688	-	70.0	110ab	53.7c	88.0	58.0	61.3
VI060794	79.5	78.5	73.7c	58.7bc	72.0	68.7	67.5
VI060818	-	77.0	76.7c	54.0c	106	80.0	63.7
P-value	0.88	0.15	0.003	0.019	0.12	0.18	0.103
Treatment and error df	5,7	10,6	11,22	11,23	11,13	10,13	10,17
F-value	0.32	2.4	3.92	2.77	1.98	1.72	1.98

Mean values with different letter(s) in a column are significantly different at $P < 0.05$.

"Blank space indicates that the variety was not tested; "-" indicates that the variety did not flower and data for commercial maturity could not be obtained. " --" indicate that the number of plants required to attaind commercial maturity (50% plants) was never reached.

- Number of pods per plant

There were significant differences among accessions in yield in Buea (P = 0.002, F = 4.82; P = 0.005, F = 4.23) and Foumbot (P = 0.0001, F = 6.12; P = 0.017, F = 2.8) during both seasons, and in Evodoula during the second season (P = 0.04, F = 2.28) (Table XXIX). VI060794 produced the highest yield in all locations and seasons than other accessions but not significantly different from the farmers' varieties, except with Gombo paysan in Buea during first season, Kirikou in Evodoula during the second season and Gombo caféier in Foumbot during the second season. The farmer varieties in all location yielded higher than most accessions but not significantly, except Gombo paysan in Buea during first (long) season and Gombo caféier in Foumbot during second season where the yield were significantly higher (Table XXIX).

Table XXIX: average number of pods per plant of the different accessions during first and second seasons 2014 at four locations

Accessions	Buea		Evodoula		Foumbot		Maroua
	First	Second	First	Second	First	Second	Second
Gombo Paysan	6.6 a	8.2 ab					
Bangourain					1.6 b	5.3 b	
Kirikou			5.5	5.7 a			
Bascko Djo							3.1
Gombo Caféier	0.0 c	7.9 ab	2.4	2.6 c	5.2 a	11.4 a	0.0
VI033805	0.0 c	3.1 bc	3.4	2.4 c	0.1 b	4.2 b	0.9
VI033824	0.7 bc	2.0 c	4.7	3.0 abc	0.5 b	4.1 b	4.3
VI036213	0.0 c	5.0 bc	6.6	3.5 abc	0.2 b	3.2 b	1.3
VI039614	1.8 bc	4.5 bc	5.7	2.8 bc	2.5 b	3.4 b	3.3
VI041210	0.0 c	2.1 c	2.3	3.2 abc	0.9 b	2.0 b	2.0
VI051114	1.8 bc	2.5 c	5.2	4.2 abc	0.1 b	3.3 b	2.8
VI057245	1.6 bc	3.1 bc	4.7	2.3 c	0.1 b	3.1 b	3.2
VI060688	0.0 c	1.5c	2.8	1.9 c	0.8 b	4.8 b	4.3
VI060794	3.9 ab	10.9 a	6.7	5.4 ab	6.7 a	5.7 b	5.6
VI060818	0.0 c	3.2 bc	5.8	1.7 c	0.9 b	4.4 b	2.1
P-value	0.002	0.005	0.13	0.04	0.0001	0.017	0.24
Treatment and error df	11,16	11,16	11,22	11,24	11,23	11,24	11,18
F-value	4.82	4.23	1.73	2.28	6.12	2.8	1.45

Mean values with different letter(s) in a column are significantly different at P < 0.05.

"Blank space indicates that the variety was not tested.

- Marketability

There were significant differences among the accessions in percentage marketable of pods only in Maroua where two of them, VI041210 and VI033805 had no marketable pods. VI039614 had the highest percentage of marketable fruits followed by VI036213 (P = 0.044, F = 2.5) (Table XXX).

Table XXX: marketable pods (%) in the different accessions during first and second seasons 2014 at four locations

Accessions	Buea		Evodoula		Foumbot		Maroua
	First	Second	First	Second	First	Second	Second
Gombo Paysan	100	100					
Kirikou			93.2	77.2			
Bangourain					90.7	100	
Bascko Djo							44.3abc
Gombo caféier	-	100	94.7	76.2	95.2	100	-
VI033805	-	100	65.5	68.2	77.8	100	0.00c
VI033824	100	100	76.3	68.2	70.8	100	56.3abc
VI036213	-	100	83.0	83.7	66.7	100	76.2ab
VI039614	100	100	100	91.1	90.0	97.2	88.9a
VI041210	-	100	62.5	59.8	14.3	100	0.00c
VI051114	100	100	85.3	70.7	100	100	25.5bc
VI057245	95.2	100	88.7	80.2	100	98.7	58.6abc
VI060688	-	100	100	81.6	91.7	99.0	29.2bc
VI060794	95.8	100	68.3	80.6	83.9	99.5	40.5abc
VI060818	-	100	100	82.1	62.5	100	54.5abc
P-value	0.55	-	0.13	0.475	0.052	0.62	0.044
Treatment /error df	5,7	11,13	11,24	11,24	11,24	11,24	10,18
F-value	0.91	-	1.72	1.00	2.19	0.82	2.50

Mean values with different letter(s) in a column are significantly different at P < 0.05.

"Blank space indicates that the variety was not tested; "-" indicates that the variety did not flower and data for marketable pods could not be obtained.

III.1.7.3. Effect of ecozones on okra pod size

- Pod length

Average pod length showed significant differences among accessions in Buea and Evodoula in all seasons, in Foumbot only during the first season and in Maroua during the second season. VI051114 had significantly higher pod length than most other ones in most locations and seasons. Where the pod length of VI051114 was shorter, it was not signficantly different from the other accessions (Table XXXI).

Table XXXI: average length of pods (cm) of the different accessions during first and second seasons at four locations in 2014

Accessions	Buea		Evodoula		Foumbot		Maroua
	First	Second	First	Second	First	Second	Second
Gombo Paysan	6.2bc	5.1cd					
Kirikou			07.8cde	5.9cd			
Bangourain					4.3ab	4.3	
Bascko Djo							15,8a
Gombo Caféier	-	4.8d	06.5de	4.9d	5.4ab	4.8	-
VI033805	-	6.5abcd	11.6abc	8.4bcd	5.0ab	7.3	4,5b
VI033824	8.8abc	5.4abcd	12.4abc	11.2ab	5.7ab	5.9	5,9b
VI036213	-	5.5abcd	14.9ab	11.7ab	6.0ab	6.4	5,9b
VI039614	6.9bc	8.7ab	15.6ab	8.7bcd	7.3ab	5.0	9,9ab
VI041210	-	5.0bcd	11.3bcd	10.9ab	8.3a	5.8	5,2b
VI051114	11.5a	6.4abcd	16.5a	13.9a	6.0ab	5.3	8,3b
VI057245	10.4ab	4.8cd	11.2bcd	9.4bc	4.1ab	4.9	6,2b
VI060688	-	8.0abc	08.0cde	8.7bcd	8.3a	3.9	11,9ab
VI060794	4.8c	4.9cd	05.7e	5.4d	4.7ab	4.7	5,7b
VI060818	-	9.0a	12.5abc	6.7cd	3.9b	5.0	7,4b
P-value	0.039	0.011	0.0002	0.0001	0.028	0.4	0,031
F-value	4.38	13.8	6.98	5.9	3.23	1.15	2,78
Treatment and error df	5,7	11,4	11,17	11,24	11,12	11,13	10,17

Mean values with different letter(s) in a column are significantly different at $P < 0.05$.

"Blank space indicates that the variety was not tested; "-" indicates that the variety did not flower and data for pod length could not be obtained.

Pod width

There were significant differences among accessions in average pod width in all seasons and locations, except in Buea during the second season (Table XXXII). Contrary to pod length, most farmers' varieties and the most susceptible okra accession (VI060794) had the highest pod width (Table XXXII).

Table XXXII: average width of pods (cm) of the different accessions during first and second seasons at four locations in 2014

	Buea		Evodoula		Foumbot		Maroua
Accessions	First	Second	First	Second	First	Second	Second
Gombo Paysan	3.1a	2.6					
Kirikou			3.7b	3.1ab			
Bangourain					2.1abc	2.2a	
Bascko Djo							2.3bcd
Gombo Caféier	-	3.2	4.4a	2.7bc	2.9a	2.2a	-
VI033805	-	2.0	2.4cd	2.8bc	1.5bc	1.8ab	1.7de
VI033824	1.8bcd	1.4	2.0d	2.0de	1.5bc	1.6ab	1.9cde
VI036213	-	1.7	2.1cd	2.1de	1.5bc	1.4b	1.7de
VI039614	1.5d	1.9	2.0d	1.9e	1.7bc	1.5ab	1.9cde
VI041210	-	2.5	2.6c	2.3cde	1.7bc	1.5ab	1.4e
VI051114	2.0bcd	1.6	2.0d	1.9e	1.5bc	1.5ab	1.7de
VI057245	2.3bc	1.6	2.2cd	2.0de	1.3c	1.2b	2.0bcde
VI060688	-	1.5	2.7c	2.4cde	2.2abc	1.6ab	2.7b
VI060794	2.7ab	2.6	3.3b	3.3a	2.4ab	2.2a	3.6a
VI060818	-	2.0	2.4cd	2.4cd	1.7bc	1.5ab	2.5bc
P-value	0.005	0.09	<.0001	<.0001	0.002	0.018	<.0001
F-value	9.71	4.19	23.66	9.5	6.39	3.51	10.03
Treatment and error df	5,7	11,4	11,17	11,24	11,12	11,13	10,17

Mean values with different letter(s) in a column are significantly different at $P < 0.05$.

"Blank space indicates that the variety was not tested; "-" indicates that the variety did not flower and data for pod width could not be obtained.

- Pod weight

There were significant differences among accessions in pod weight only in Evodoula during the second season (P = 0.004, F = 3.57). In this location, VI051114 had the highest pod weight than most accessions and farmers' varieties (Table XXXIII).

Table XXXIII: average weight per pod (g) of the different accessions during first and second seasons at four locations in 2014

Accessions	Buea		Evodoula		Foumbot		Maroua
	First	Second	First	Second	First	Second	Second
Gombo Paysan	19.4	10.0					
Kirikou			15.7	7.9bcd			
Bangourain					5.8	2.9	
Bascko Djo							17.6
Gombo Caféier	-	7.0	26.3	7.1cd	10.7	3.2	-
VI033805	-	4.5	16.2	11.4abc	8.0	1.0	06.6
VI033824	11.1	5.0	14.6	11.7abc	9.1	3.0	10.7
VI036213	-	6.0	20.0	13.3ab	7.0	0.3	09.6
VI039614	4.4	11.3	19.5	5.7cd	8.2	0.3	12.9
VI041210	-	7.0	19.8	11.3abc	7.3	0.5	03.5
VI051114	23.5	4.5	16.8	16.4a	7.0	1.0	14.5
VI057245	18.4	4.0	13.0	9.7bcd	4.5	0.3	10.7
VI060688	-	6.0	14.3	5.1d	18.5	1.5	23.1
VI060794	13.8	9.0	11.4	7.8bcd	14.3	2.6	22.0
VI060818	-	6.0	08.5	6.9cd	7.4	0.9	30.9
P-value	0.075	0.2	0.219	0.004	0.062	0.156	0.56
F-value	3.32	2.8	1.5	3.57	2.54	1.62	0.89
Treatment and error df	5,7	10,12	11,17	11,24	11,12	11,24	10,18

Mean values with different letter(s) in a column are significantly different at P < 0.05.

"Blank space indicates that the variety was not tested; "-" indicates that the variety did not flower and data for pod weight could not be obtained.

III.1.7.4. Plant vigour

- Plant leaf area

Plant leaf area only showed significant differences among accessions during the second season at Evodoula (P = 0.045, F = 2.27) and first season in Foumbot (P <.0001, F = 6.49) (Table XXXIV).

Table XXXIV: mean plant leaf area (cm^2) of the different accessions during first and second seasons at four locations in 2014

	Buea		Evodoula		Foumbot		Maroua
Accessions	First	Second	First	Second	First	Second	Short
Gombo Paysan	181.8	149.5					
Bangourain					92.0b	29.7	
Kirikou			178.5	77.8abc			
Bascko Djo							275.9
Gombo Caféier	174.1	82.9	120.0	83.5abc	139.4a	26.2	190.2
VI033805	83.1	76.4	277.4	119.8a	62.6bcd	29.2	210.3
VI033824	91.5	54.3	155.7	59.7bc	62.8bcd	36.9	142.0
VI036213	24.4	165.9	188.5	69.6abc	58.3bcd	16.6	211.5
VI039614	23.3	41.1	141.0	35.6c	73.9bc	15.7	250.3
VI041210	111.3	162.6	239.7	105.4ab	87.8bc	27.5	163.3
VI051114	112.3	162.1	182.9	67.6abc	32.8d	20.3	202.5
VI057245	105.8	50.5	150.0	45.5c	39.6c	16.1	214.7
VI060688	13.2	40.4	134.6	58.3bc	55.1bcd	20.6	263.0
VI060794	95.2	149.6	157.1	63.1bc	148.6a	29.2	257.6
VI060818	79.0	65.9	201.5	46.7c	62.0bcd	19.3	169.2
P-value	0.436	0.198	0.56	0.045	<.0001	0.18	0.525
F-value	1.05	1.6	0.89	2.27	6.49	1.54	0.94
Treatment and error df	11,24	11,14	11,22	11,24	11,23	11,24	11,21

Mean values with different letter(s) in a column are significantly different at P < 0.05.

“Blank space indicates that the variety was not tested

- Plant height

Plant height revealed significant differences among accessions at Buea in all seasons ($P < 0.0001$, $F = 7.26$ and $P = 0.025$, $F = 3.46$), during the first season in Foumbot ($P < 0.0001$, $F = 7.84$) and during the only season in Maroua ($P < 0.0001$, $F = 11.82$). In these cases, all farmers' accessions had the highest plant height, but not significantly different from Gombo caféier and VI060794 in Buea during first and second season respectively, in Foumbot during the first season and only VI060794 in Maroua (Table XXXV). Where there were significant differences, all the farmers'varieties had significantly higher plant height.

Table XXXV: mean plant height (cm) of the different accessions during the first and second seasons at four locations in 2014

	Buea		Evodoula		Foumbot		Maroua
Accessions	First	Second	First	Second	First	Second	Second
Gombo Paysan	87.8a	45.5a					
Bangourain					59.9a	25.9	
Kirikou			55.7	41.4			
Bascko Djo							46.4a
Gombo Caféier	77.5a	20.3b	77.0	60.0	44.7ab	28.2	31.1b
VI033805	29.5b	26.6b	92.1	54.7	22.3cd	26.0	30.3b
VI033824	28.2b	31.0b	58.1	26.3	19.9cd	32.2	17.9d
VI036213	30.3b	17.5b	72.4	35.6	18.1d	23.4	20.9cd
VI039614	28.8b	17.5b	60.0	23.6	30.8bcd	21.0	32.3b
VI041210	27.1b	19.0b	58.5	49.8	16.9d	24.6	18.9cd
VI051114	45.1b	23.5b	66.2	38.3	18.9cd	22.9	24.8bcd
VI057245	29.4b	17.5b	54.7	32.8	15.3d	25.4	25.0bcd
VI060688	29.3b	20.0b	53.8	28.1	23.0cd	26.5	27.5bc
VI060794	44.0b	36.5ab	68.4	45.1	45.7ab	26.3	43.4a
VI060818	41.7b	26.7b	87.1	34.9	35.6bc	26.5	30.0b
P-value	<.0001	0.025	0.382	0.09	<.0001	0.91	<.0001
F-value	7.26	3.46	1.14	1.89	7.84	0.45	11,82
Treatment and error df	11,24	11,11	11,20	11,24	11,23	11,24	11,19

Mean values with different letter(s) in a column are significantly different at $P < 0.05$.

"Blank space indicates that the variety was not tested

- Plant stem diameter

Stem diameter varied significantly among accessions in all locations and seasons, except during the second season in Foumbot. VI060794 had higher stem diameter at least during one of the seasons in all locations. Gombo caféier had similar results but not in Maroua (Table XXXVI).

Table XXXVI: mean plant stem diameter at 5 cm from ground level for the different accessions during the first and second seasons at four locations in 2014

Accession	Buea		Evodoula		Foumbot		Maroua
	First	Second	First	Second	First	Second	Second
Gombo Paysan	2.2b	1.2ab					
Bangourain					2.1a	0.9	
Kirikou			1.2bcd	3.3abcd			
Bascko Djo							1.2bcd
Gombo Caféier	3.2a	1.3ab	1.8ab	4.2a	1.7a	0.8	1.5bc
VI033805	1.5bc	1.1ab	1.9a	4.3a	1.1b	0.4	1.6b
VI033824	1.2c	1.5ab	1.1cd	3.2abcd	0.8bc	0.7	0.8d
VI036213	1.1c	1.0ab	1.6abc	3.7abc	0.9bc	0.5	1.0d
VI039614	0.9c	0.6b	0.9d	2.1d	0.6bc	0.6	1.2bcd
VI041210	1.6bc	0.7b	1.7abc	4.3a	0.9bc	0.7	1.0d
VI051114	1.7bc	1.2ab	1.2bcd	3.4abcd	0.7bc	0.6	1.2bcd
VI057245	1.2c	0.8ab	1.1cd	2.6bcd	0.6c	0.5	1.1cd
VI060688	1.3bc	0.5b	1.0d	2.3cd	1.0bc	0.5	0.9d
VI060794	1.8bc	1.8a	1.8ab	3.9ab	2.0a	0.6	2.2a
VI060818	1.2c	0.7b	1.3abcd	2.6bcd	0.8bc	0.5	1.0d
P-value	0.001	0.006	0.006	0.007	<.0001	0.75	<,0001
F-value	4.45	4.7	3.66	3.28	16.17	0.67	8,73
Treatment and error df	11,24	11,12	11,20	11,24	11,23	11,24	11,19

Mean values with different letter(s) in a column are significantly different at $P < 0.05$.

"Blank space indicates that the variety was not tested

III.2. Discussion

Genetic studies on the populations of *Aphis gossypii* from Cameroon and Taiwan, with an Indian reference population, showed that this arthropod is a common aphid species on okra in these countries; no other species was identified during the study. Two biotypes (melon and cotton) of *A. gossypii* were recognized (Guldemond *et al.*, 1994). Although they are morphologically indistinguishable, they have distinct host ranges. In addition, some differences in host preference (Wang *et al.*, 2004) and feeding behaviour (Gutierrez *et al.*, 2008) exist between these melon and cotton biotypes. Crop resistance to *A. gossypii* also has been shown to be biotype-specific (Dogimont *et al.*, 2008). Up to now, there have been no reports about the occurrence of *Aphis gossypii* biotypes on okra. In the present study, it appears that *A. gossypii* individuals attacking okra in Cameroon, India and Taiwan are not genetically diverse. Hence, okra accession(s) with appreciable levels of aphid resistance in Taiwan may react the same way in Cameroon, unless environmental factors alter the resistance reactions.

The okra accessions screened in this study ranged from highly resistant to highly susceptible to aphids. The diversity of origins and high number of accessions used increased the chances of obtaining sources of resistance to aphids. This study included several popular okra varieties collected from farmers' fields in Cameroon, which were mostly *Abelmoschus caillei*. However, none of the varieties obtained from farmers was resistant to *A. gossypii*. This indicates that in Cameroon, this insect is a serious pest of okra, and that the varieties cultivated by farmers are highly proned to aphid infestation. These crops produced higher number of pods per plant (5 to 13 pods per plant) than the other resistant accessions (0.9 to 4 pods per plant) that were screened together. However, the farmers' varieties had about 60 to 100 days to 50% anthesis, as compared to 40 to 60 in the other ones. At the end of the present study, nine okra accessions out of over 445 were identified as resistant or moderately resistant to *A. gossypii* after screening. These are VI051114, VI036213, VI033805, VI041210, VI060688, VI060818, VI039614, VI033824 and VI060794. Three of them (VI051114, VI036213 and VI033805) were rated as resistant to aphid earlier during this study. VI051114, known as Utong, was collected during 2002 from La Union Province (16°45'07.7"N 120°28'22.6"E) in the Philippines where it is considered a local variety. VI036213 was collected during 1991 from Rizal Province (14°31'N 121°15'E) in the Philippines, and VI033805 was collected during 1991 from Ilocos Sur Province (17°20'N 120°30'E) in the Philippines. VI036213 and VI033805 are considered as old cultivars

as farmer are now trying new ones there. Six more accessions: VI041210, VI060688, VI060818, VI039614, VI033824 and VI060794 were identified later during the present study. Although VI057245 was included as the known susceptible accession, it was constantly being moderately resistant in both the trials in Cameroon and the final trial in Taiwan. VI060794 was later on added to the list due to its high susceptibility in one of the final trials in Taiwan as well. It should also be noted that VI060794 and VI057245 are not highly susceptible accessions, for they showed a moderate resistance in some trials. Since this was a newly initiated aphid-resistance screening program involving the okra germplasm from AVRDC – The World Vegetable Center, and we did not have a well-known aphid susceptible okra line, we chose susceptible checks from the available list. The most common varieties Kirikou and Gombo caféier cultivated in Cameroon in general and in Yaoundé particularly were used as farmers' checks. Kirikou was the most susceptible while Gombo caféier was one of the most resistant. The inconsistency in the resistance performance of the accessions, particularly VI057245, suggests that resistance to aphids may vary in space and time. Nevertheless, this inconsistency may help to identify consistent accessions with potential for resistance to the aphid. VI041210 was an old cultivar collected at Cabaroan (17°39' N, 120°22' E), Santa Catalina, Ilocos Sur province in the Philippines. VI057245 was a landrace collected at Cham Ko Louk (13°26'56.1" N, 103°00'13.5"), Banteay Meanchey province in Cambodia. VI060818 was a local cultivar (ORS 354) in Mali, and VI039614 was collected from Maidagiri province in the Ranchgarh state of Bangladesh, whereas VI033824 was an old cultivar collected at Koronadal, Barrio (06°30' N, 124°50' E) in South Cotabato province of the Philippines. The West African VI060794 was a local cultivar (ORS 383) cultivated in Ivory Coast and VI060688 was a local cultivar (Pusa Red) collected from Kerala in India. All these accessions were the Asian okra species of *Ab. esculentus* except, VI060794 (*Ab. caillei)* and VI060818 (*Ab. esculentus)* that were from West Africa. The farmer check used was Kirikou (*Ab. esculentus*) and the seeds were obtained from local farmers. It is important to note that Kirikou appeals to producers through its essential advantages: high productivity with many fruits from the base of the plant, a short cycle (40 to 45 days) from transplanting to the first harvest, and a thick skin, a guarantee of a good amount of mucilage. This variety distributed by SEMAGRI is the only found in all markets in Yaoundé city. Kirikou mostly cultivated in the Central Region was generally more susceptible to aphids than all accessions, except VI051114.

This indicates that *A. gossypii* is an important pest of okra in farmers' fields in Cameroon and particularly in forest zone of the Central Region.

Three of the selected accessions were susceptible in the first season and seven in the second one during the confirmatory screening. This indicates that resistance of okra to *A. gossypii* varies with time and space. The farmers' check Kirikou was the most tolerant since it was more susceptible than VI060794 that had similar and highest yield. Resistant accessions were less yielding than susceptible ones. In Cameroon, *Ab. caillei* is the most cultivated species. VI060794 was the only cultivar of *Ab. caillei* and the most infested by aphids, among the identified nine and the most tolerant like Kirikou. More than 20 accessions of *Ab. caillei* were screened during the present study, which included two commercial varieties (Gombo caféier and Gombo paysan). They were more susceptible to aphids, except Gombo caféier that was moderately resistant and highly productive. Thus, Gombo caféier, VI041210 and VI067245 were the most resistant accessions.

Plants resistance to herbivores can be conferred in three ways: antixenosis (non-preference), antibiosis and tolerance (Painter, 1951). *Ab. caillei* has been reported to be better adapted under humid zone and tolerant to biotic stress (Siemonsma, 1982). This confirms the higher level of tolerance as a resistance category of VI060794 than any other accession. The expression of tolerance is determined by the inherent genetic ability of a plant to outgrow an insect infestation or to recover and add new growth after the destruction or removal of damaged tissues (Smith, 1989). Factors affecting tolerance include plant vigour and regeneration of damaged tissues (Metcalf and Luckman, 1994). During the second season, yields were generally low because they were planted in September when the rains were very heavy and affected growth during the seedling stage of the plant; this suggests that proper timing for planting is needed to avoid climate unfavourable cultivation. However, VI041210 that produced the lowest yield during the first season, even with well-developed plant parameters, produced the highest yield during the second season. Therefore, it is known that the growth and development of okra can be affected by seasons (Siemonsma and Hamon, 2004; Siemonsma and Kouamé, 2004). Aphid infestation did not affect the yield of the susceptible okra accessions and *vice versa*. This indicates that the type of resistance to aphid found in these okra accessions is tolerance. VI060794 was the highest yielding among the accessions, and was moderately resistant during the first season. VI060688, VI036213, VI057245 and Gombo caféier were either resistant or

moderately resistant during the two seasons. VI033805, VI060818, VI033824, VI039614, VI051114 and VI041210 were not constant in susceptibility or resistance. Although the yield of these nine accessions was lower than that of the susceptible farmers' check Kirikou, they could yield better if they were adapted, as they often experience flower abortion and attack by wet rot, constraints that are not common with Cameroonian and West African varieties. Resistance through tolerance may be achieved through higher plant vigour. Vigorous plants harbour more pests and provide more food for insect development, growth and reproduction. Plant vigour compensates for insect feeding damage and consequently ensures good crop yields, thereby augmenting the crop's tolerance to pests (Ndemah, 1999; Ndemah *et al.*, 2003; Chabi-Olaye *et al.*, 2005). It is important to consider yields of host plants when identifying okra germplasm resistant to *Aphis gossypii*. VI041210 and VI060794 are potential sources of aphid-resistant and tolerant trait respectively. Kirikou was only vigorous in leaf area that was larger, accommodating more aphids. But a very good productivity with many fruits from the base of the plant, a short cycle (40 to 45 days) from transplanting to the first harvest give it the ability to escape yield loss from aphid infestation. VI041210 was more vigorous in plant and leaf size, VI060794 and Gombo caféier in plant and pod size and VI051114 in pod size.

In this study, non-preference was not a resistance category. No report exists for antixenosis in okra for aphid, but Hegde *et al.* (2012) reported that aphid antixenosis in cotton (same botanical family as okra), the primary host of *A. gossypii,* is activated by the natural plant defence elicitor cis-jasmone. The settling behaviour study revealed that *A. gossypii* did not discriminate between the resistant and susceptible okra accessions under choice condition, and that there were no significant differences among accessions for aphid permanence on infested leaf; this suggests that the phenotypic structures and allelochemicals had little or no effect on the settling behaviour of aphids on okra. In other plant families, mostly melon family, antixenosis of the cotton aphid (*A. gossypii*) was demonstrated (Garzo *et al.*, 2004 and Moghadam *et al.*, 2013), because of the presence of "Vat" and "Agr" genes and leaf pubescence respectively. However, as also demonstrated in the current study, Sarria *et al.* (2010), who worked on role of leaf glandular trichomes of melon plants in deterrence of *A. gossypii,* did not find aphid antixenosis. In phytophagy, there exist three stages of interaction: pre-entry, entry, and colonization (Walling, 2008). Allelochemical and leaf chorophyll may influence the first stage of this interaction, which is pre-entry or settling behaviour. The non-discrimination between susceptible and resistant

accessions in aphid settling behaviour indicates that allelochemicals and leaf chorophyll did not influence attraction. The second stage of plant-biotroph interaction, which is entry and feeding, can be influenced by leaf trichomes and leaf toughness. Trichomes have either adverse (Zarpas *et al.,* 2006) or positive effects (Nibouche *et al.,* 2008) on resistance to *A. gossypii*. Our study revealed that trichome density on leaves from the middle and bottom strata did not vary significantly among the okra accessions. However, the trichome density in the younger leaves of VI033805 (which had the lowest aphid infestation than VI057245, VI051114 and VI036213) was significantly higher. Further studies on leaves trichome density involving these four accessions and five additional selected aphid-resistant accession and susceptible checks revealed lower trichome densities in two of the susceptible checks (VI060794 and Kirikou), at all plant strata. Other authors also found that the leaf trichome density in okra affects *A. gossypii* infestation (Santos *et al.,* 2003; Soglia *et al.,* 2002 and 2003 and Leite *et al.,* 2007). In the present study, the trichome density was highest for the leaves of the top stratum, higher for the middle stratum and lower for the bottom. In the field, aphids would have the choice to colonize leaves that have lower trichome density thus avoiding the need of attacking the younger ones. A higher density of trichomes has been observed in the apical part than in the middle and bottom parts of okra (Leite *et al.,* 2007). In the present study, spatial distribution of aphids showed that aphids infest mostly leaves of the middle and bottom strata of the plant than the top stratum and the apex. Some studies showed that *A. gossypii* populations prefer young and succulent leaves, which are generally located on the apical parts of susceptible varieties (Santos *et al.,* 2003; Chau *et al.,* 2005). Non-occurrence of aphids on the apical leaves of resistant varieties is an indication that these leaves have more trichomes, which can make locomotion, feeding and reproduction of *A gossypii* difficult (Soglia *et al.,* 2002 and 2003; Santos *et al.,* 2003; Leite *et al.,* 2007). This has been confirmed in the present study, and recently by Tan *et al*. (2012). Since the trichome density in the leaves of the least infested okra accessions or most resistant accessions such as VI041210 and VI057245 were significantly higher, it could be suggested that the physical effects of pubescence may influence infestation by *A. gossypii* in okra. Leaf trichomes are one of the factors that influence the colonization of okra plants by this arthropod species. Gombo caféier had low trichome density but it was one of the most resistant varieties probably due to relative leaf hardness. Although the differences in leaf trichome between the resistant accessions and susceptible checks did not provide any antixenotic effects in the resistant accessions, high

trichome density may play a key role in reducing feeding and oviposition in resistant accessions, while low trichome density in susceptible check may favour feeding and oviposition and eventual population build-up. In addition to trichomes, leaf toughness is important in enhancing plant resistance (Deguine and Hau, 2001). However, our current study did not find any evidence supporting the influence of leaf toughness against aphid infestation on resistant or susceptible accessions.

Apart from a biophysical characteristic such as leaf trichome, plant chemistry also affects pest infestations, mostly the third stage of plant-biotroph interaction, which is colonization. The most susceptible accessions had significantly lower leaf trichome density. This characteristic is probably responsible for the higher colonization of susceptible okra accessions. Plant nitrogen is an indicator of food quality and host selection by *A. gossypii* (Mattson, 1980; Slosser *et al.*, 1989) although Leite *et al.* (2007) argued that leaf nitrogen and organic compounds in okra did not have any effects on *A. gossypii* populations. In addition, Lu *et al.* (2009) found that total nitrogen and amino acids in cotton were not associated with resistance to *A. gossypii*. On the contrary, the current study revealed the role of total nitrogen leading to the susceptibility of okra accessions to aphids. An excess of nitrogen (N) or deficiency of potassium (K) can lead to higher accumulation of amino acids, which in turn can cause higher attack rate by sucking insects (Jansson and Ekbom, 2002). Nitrogen level was significantly higher in leaves of the susceptible VI060794 than in the other less susceptible accessions. These parameters did not affect aphid settling behaviour, but could be responsible for subsequent population build-up on the susceptible accessions (Lazzari and Zonta-de-Carvalho, 2012). Sugars are necessary for the normal growth and development of insects, and their concentration in host plants favours feeding by insects. Infestation by *Aphis gossypii* was higher on accessions with higher total sugar concentration of okra leaves. In contrast, Deguine and Hau (2001) observed that the sugar content was twice as high in *A. gossypii*-susceptible cotton leaves. However, the effect of reducing sugars was less significant in leafhopper-resistant okra varieties (Singh and Agarwal, 1988). Similarly, the sugar content did not influence the thrips population in onion (Saxena, 1970). It was thus not a surprise to find that the sugar concentration of okra leaves did not influence *A. gossypii* feeding behaviour. Plant nutrients such as carbohydrates and proteins (amino acids) are also stated important for the development of *A. gossypii* (Slosser *et al.*, 1989). However, many authors have shown contradictory results. For instance, leaf nitrogen and organic compounds in okra did not have any

effects on *A. gossypii* populations (Leite *et al.,* 2007). According to Lu *et al.* (2009), total nitrogen and amino acids in cotton were not associated with resistance to this arthropod species. Zucker (1982) found an inverse correlation for the effect of total phenols in *Populus anqustifolia* to the galling aphid, *Pemphigus betae*. The tannin content did not vary among the resistant and susceptible okra accessions. Tannins are believed to offer protection against phytophagous insects by reducing digestibility, but the defensive effects of tannins cannot be generalized. The average probing duration and the total probing time of *A. gossypii* fed on an artificial diet containing tannic acids were shown to be significantly reduced (Ma *et al.,* 2005). Lu *et al.* (2009) found that tannin content was negatively correlated with *A. gossypii* resistance in cotton. On the contrary, Zucker (1982) demonstrated an inverse correlation for the effect of total phenols in *Populus anqustifolia* to a galling aphid, *Pemphigus betae*. Similarly, preliminary results during the current study revealed that total phenols were lower in some resistant accessions. Plants of different ages may vary in the quantities of nutrients and toxins they contain (Singh and Sinhal, 2011). Since the total nitrogen content was significantly different between the susceptible and most okra accessions with lowest aphid infestation, it was concluded that the high leaf nitrogen content has a positive effect on the host selection process of *A. gossypii*.

Some traits and processes that defend plants against pests change following pest attack, as induced response to attack and damage (Khattab, 2007; Wilson *et al.,* 2011) or following crop phenology. In the current study, biochemical studies of selected okra accessions at six and 10 weeks after sowing showed changes in leaf tannins following attack. When there was an aphid infestation, the plant defence compounds, especially phenolic compounds, were increased. Tuomi *et al.* (1988) recorded a similar rapid increase in total phenols following damage. It seemed that aphid feeding induced defence since the total tannins increased in the selected accession and reduced in the susceptible farmer's check at all plant growth stages, corroborating the result of Ma *et al.* (2005) who found that tannins affected *A. gossypii* feeding. The increase in total phenols following an attack was a general tendency by all germplasms to offer resistance to aphid feeding. Total sugars and reducing sugars may also have a significant role in offering resistance in plants infested with or without aphids at all growth stages. Studies on the first (2011) and second (2012) selections showed that, at reproductive stage (10 WAS), total sugars were also significantly higher in susceptible Kirikou while reducing sugars increased by more than 100%, and Potassium content decreased following attack more than in the resistant accessions. Excess of

nitrogen (N) or deficiency of potassium (K) can lead to higher attack rate by sucking insects. For the accessions selected in 2013 (third selection), after infestation, VI057245 that had significantly higher Potassium content than most accessions was recorded lower aphid population build-up. At 10 weeks after sowing, it was significantly higher in the two most resistant accessions (VI057245 and VI041210) than in the other moderately resistant accessions, when infested with or without aphids. Total nitrogen at all growth stages were higher in the susceptible VI060794 than the other accessions, confirming that leaf nitrogen content had a positive effect on the host selection process of *A. gossypii*. Nitrogen is frequently considered as a limiting resource for insects (Mattson, 1982; Bernays, 1992). The leaf nitrogen content is generally accepted as an indicator of food quality (Scriber and Slansky, 1981) and as a factor affecting host selection by phytophagous insects (McNeill and Southwood, 1978; Mattson, 1982; Bernays, 1992). Nitrogen deficiency in plant is indicated by chlorosis. As a susceptible accession, VI060794 had significantly higher leaf nitrogen at vegetative stage following aphid infestation, and also at reproductive growth of the plant even when plants are not infested, making this accession a suitable source of nitrogen to *A. gossypii.* The farmer's variety Kirikou had lower nitrogen content probably because aphids feed more on it and reduce accumulation of nitrogen, while low leaf tannins could have contributed to its susceptibility than in selected resistant accessions at both vegetative and reproductive stages of plant growth, and when the plants were previously infested with aphids before sampling. This result corroborated the findings of Khattab (2007) and Wilson *et al.* (2011) that some of the traits and processes that defend plants against pests change following attack as induced response to infestation and damage. Induced responses that reduce herbivore survival, reproduction or preference for a plant are termed induced resistance. Phenols, most abundantly tannins act as toxins, repellents, bind insect salivary proteins (Chandramani et al., 2009), resulting to a defence category called antibiosis. Painter (1951) stated that antibiosis refers to the adverse effects on insect life history when a resistant plant variety is used as a food source. life history of an insect is made up of its growth, development and reproduction.

Capinera (2005) described the biology of the cotton aphid, *A. gossypii* Glover, and gave the duration of adult's reproductive period as about 15 days, four moults per nymphal period, and duration of 1 to 3 days per moult. These values vary considerably, mostly as a function of temperature. The optimal temperature for reproduction is reported to be about 21 °C to 27 °C. Females produce about 70 to 80 offsprings at a rate of 4.3 per day. These results were obtained in

some accessions in the present study, except the intrinsic rate of increase that was less than 4.3 in all situations. Only VI033805 and VI033824 had number of offsprings per adult ranging from 70 to 80. Several studies have reported results similar to those of the present study in the life table parameters of *A. gossypii* on okra (Agarwala and Das, 2012; Satar *et al.*, 1999 and Satar *et al.*, 2013).The farmers check Kirikou and VI051114 were the only accessions susceptible across the two seasons.

At the reproductive stage of the plant, aphid development was the same in all accessions. In the third selection, at vegetative stage of plant growth, although the number of moults was only significantly higher with VI060818 than with VI057245, the fourth instar duration was significantly longer with VI060818 than with VI039614; the generation time (To) was also significantly longer with VI057245 than with three accessions (VI041210, Kirikou and Gombo caféier). The average duration per moult was not significantly different in all accessions. Thus, development of aphids in the third selection was constitutively not a mechanism of resistance. Similar results were obtained at the reproductive stage of plant growth in this third selection, but the possible reason for the susceptibility of the farmers check could be the duration per moult that was shorter as well as with VI060818. The susceptibility of VI051114 and Kirikou and moderate susceptibility of VI033805 could be due to rapid aphid development as shown during vegetative growth of plant of first and second selection not previously infested with aphids. The other accessions, namely Gombo caféier, VI041210, VI036213, VI039614, VI060818, VI060688, VI057245, VI033824 and VI060794, could achieve resistance through poor nymphal development. Poor development of a pest on host plants can only lead to host plant resistance if the survival and reproductive performance of the pest are affected negatively. In the present study, the poor development of the pest on some accessions without previous infestation of plants with aphids led to negative reproductive performance of the pest during the vegetative stage of the plant growth. The farmer's check Kirikou had the highest intrinsic rate of natural increase, which was significantly different from VI057245, one of the most resistant accessions. Thus, the reproductive performance of *A. gossypii* was higher on the susceptible Kirikou and lowest on VI057245.

When plants were previously infested with 25 to 35 aphids at vegetative growth and 100 to 200 aphids at reproductive stage, the developmental time of aphid was significantly longer with VI041210 than all accessions, except with VI060688 at vegetative growth. VI041210 was

the only accession that was resistant in the first season and the only most tolerant accession in the second season. This result is an evidence of response to feeding by aphids that induced defence against *A. gossypii*. The induced defence was found since no mortality of aphids was observed on the farmers' check Kirikou, VI033805 and VI033824, significantly lower than that of aphids feeding on the other varieties and especially on VI041210, one of the most resistant accessions, where development (nymphal) time was the longest. It seemed that there was antibiotic property of tannins in the accessions with higher mortality during this vegetative stage of plant growth, since it was only at this stage that there were significant differences among accessions in nymphal mortality. Tannins are believed to offer protection against phytophagous insects by reducing digestibility; however, the defensive effects of tannins cannot be generalized. It seemed that tannins reduced the average probing duration and the total probing time by *A. gossypii* fed on VI041210, as suggested by Ma *et al.* (2005) working on total probing time of this pest feeding on a diet containing tannic acids. At reproductive stage, no clear effect between the resistant and susceptible accessions was shown on the reproductive performance of aphids. Although the evidence of induced response found in VI041210 did not lead to poor reproduction in this accession at this stage, the mortality was at least higher but not significantly with resistant VI041210 and lower with VI060794 which was the susceptible check leading to antibiosis. In addition, reducing sugars were higher in VI041210 at reproductive stage than at vegetative stage with respect to other accessions, and most have favoured reproductive performance of aphids on this accession. Furthermore, the presence of largest leaf sizes of VI041210 that could harbour more aphids could have obscured the poor reproduction of aphids on VI041210 compared to other resistant entries. Previous infestation offered resistance to aphids in VI041210, thus secondary infestation is irrelevant in this entry since its resistance is induced by aphid feeding, while primary infestation is irrelevant in VI057245 and Gombo caféière that have constitutive resistance. Apart from VI041210, two varieties VI036213 and VI033824 whose resistance increased at plant maturity, witnessed an increase in total phenols at the reproductive stage. Recently Agarwala and Das (2012) reported that aphids perform poorly on mature plants. This could only be true with some accessions and not with others since we face contradictory results, such as that of Anitha and Nandihalli (2008) suggesting that plants nearing maturity showed more susceptible than other stages for these sucking pests. Nevertheless, the fact that the more susceptible accession exhibited this tendency the most is an indication that the less susceptible

accessions may have some resistance to *A. gossypii*. The biological cycle of aphids on Kirikou was longer than on resistant varieties, while the intrinsic rate of increase and finite rate of increase was higher; the population doubling time was significantly shorter than on resistant VI057245 at vegetative stage of uninfested plants. This trend was not observed at reproductive stage but susceptible VI060794 had highest intrinsic rate of increase and finite rate of increase, suggesting that VI057245 has antibiotic properties. However, the role-played by phenotypic structures and allelochemicals to aphid development and reproduction vary depending on accession, crop phenology and infestation levels.

In June, the aphid population decreased only on VI041210 and VI036213, while all the other accessions were able to attain the lowest pupolation at one point during the season except VI051114. Population on the two local varieties (Kirikou and Gombo caféier) maintained an intermediate level throughout the season. VI057245 and VI060794 had their lowest infestation at the begining which was steadily rising till the end of the season. In the second season, aphid population dynamics had uniform trend in all accessions. The resistance or susceptibility of VI041210, VI033824 was not constant throughout the season. The two most susceptible accessions (VI060794 and Kirikou) maintained higher aphid populations throughout the second season. They have either maintained relative higher aphid infestation or a steady rise during both seasons. Apart from crop phenology and its effects on crop resistance, seasonality can influence aphid population dynamics. The general rise in aphid population in April and June during the first season and the peak in late October and early December and during the second season indicate that, the peak infestation period for aphids on okra may be April and June during the first season and October and December during second season. These results corroborate the studies done by Anitha and Nandihalli (2008). Similar trends were also reported by Senapathi and Mohanty (1980), Patel and Rote (1995), Hegde *et al.* (2004) and Gulati (2004). During our study, the rise in infestation by aphids from June indicates that another peak might be in July as reported by Dugger and Richter (1998) for aphid infestation on cotton. Mahmoot *et al.* (1990) found similar trend on a single season with two serious aphid activity peaks during early June and from late September to early October. However, the aphids did not remain serious problem throughout the growth period, as was the case with VI060794 and Kirikou especially during the second season during the confirmatory screening. Srinivasan and Krishnakumar (1983) reported the same pest as a serious pest throughout the cropping season. As earlier mentioned, the slight difference with the

current study could be due to differences in climatic conditions. For aphid population dynamics on the three most resistant accessions during first season, infestation on VI041210 decreased while on VI057245 it started with low infestation which increased steadily and attained an intermediate level. The third resistant variety "Gombo caféier" maintained an intermediate level of infestation throughout the season. During the second season, these resistant ones (VI041210, VI057245 and Gombo caféier) maintained lower infestations than the two most susceptible ones (VI060794 and Kirikou), throughout their growth. Nevertheless, the fact that only three varieties were identified out of 445 seems to prove that sources of resistance of okra to *A. gossypii* are limited. These two most susceptible varieties were the most productive hence; tolerance is an obvious category of resistance. Accessions with steady rise in aphid population may need chemical control each time the population is above the economic threshold level. Therefore, effective control of this pest on okra should not only depend on host plant resistance. The limitations of tolerant accessions cannot be over emphasized. Accessions with low infestation at anytime during the season may not need chemical control. Nevertheless, the younger and tender crop may need more attention to ensure good growth and acceptable yield, while at maturity infestation might not affect the yield significantly. For effective control of the pest on okra, careful scouting at regular intervals throughout the growth period of the crop is essential to determine whether insecticide spray is needed or not.

The performance of the nine okra accessions identified for resistance to aphids can vary between seasons and locations. Therefore, it is important to evaluate their adaptation to Cameroon's agro-ecological conditions to confirm their yield potential before recommending them for farmers' use. Results from the multi-location screening trials indicated that the varieties used by farmers in Cameroon were more susceptible to aphids than most of the selected resistant accessions across all agro-ecological zones. However, the common commercial variety "Gombo caféier" showed some resistance to *A. gossypii* in Evodoula in all seasons and in Buea during the second season, and in the semi-arid zone. However, the resistance was consistent with VI036213 in Foumbot, VI039614 in Buea, and VI051114 in Evodoula. In all the locations, the resistance of VI060794 was consistent except during the first season at Foumbot and in Maroua. The trial in Maroua, which is a semi-arid region was conducted only once from September to December 2014, where aphids did not infest two accessions (VI041210 and VI060818). The farmer variety from this region (Bascko Djo) was rated susceptible compared to the selected accessions, except

VI060794. The higher susceptibility of each farmer's variety in each location confirmed the importance of aphids as pest in Cameroon. The resistance in the selected accessions also varied with time and space. Aphid infestations were higher in the second seasons than in the first in Evooula and Foumbot but not in Buea. Similar trends were observed during confirmatory screening, where three of the selected accessions were susceptible in the first season and seven in the second season. High rainfall is an important mortality factor of *A. gossypii* in the field (McDonald *et al.*, 2003; Rhainds and Messing, 2005). Anitha (2007) reviewed climatic factors and found temperature as one of the factors affecting aphid population in the field. Seasons may have had an effect on resistance since this resistance changed positively in some accessions and negatively in others, despite the lower rainfall and higher temperatures observed during the second seasons. VI060794 and VI036213 possessed resistance to aphids in the western highland, VI060794 and VI039614 in the monomodal humid rain forest, Gombo caféier, VI060794 and VI051114 for the bimodal humid rain forest and VI060794, VI060818, and VI041210 in the Sudano-Sahelian region.

All these accessions were the Asian okra species *Ab. esculentus,* with most of the accessions originating from the Philippines, except VI060794 and VI060818 from West Africa. VI060794 and VI060818 are *Ab. caillei* (West African okra) and *Ab. esculentus* (common okra), respectively. It is only in West and Central Africa (accounting for about 10% of the world's production) that common okra and West African okra are both used. They now share the market roughly fifty-fifty (Siemonsma and Hamon, 2004; Siemonsma and Kouame, 2004) as *Ab. caillei* has gradually replaced common okra in the tropical-humid regions because of its better adaptation under humid zone and tolerance to biotic stresses (Siemonsma, 1982). VI060794 produced the highest yield in all locations and seasons than other accession, but not significantly different from the farmers' varieties, except with Gombo paysan in Buea during first season and Gombo caféier in Foumbot during the second season, where their yields were not significantly different from that of VI060794. These three are all *Ab. caillei*, thus it confirmed the fact that this species is more adapted and more tolerant. However, under very limited and erratic rainfall, especially in the semi-arid zones, earliness of *Ab. esculentus* (being amphidiploid) as compared to *Ab. caillei* (being amphipolyploid) is preferred. Significant differences among accessions in days to commercial maturity were observed only in Evodoula in both seasons, where Gombo caféier had the longest duration of commercial maturity in both seasons. This duration was also either

higher or infinitive during the first season probably due to the effect of season on flowering. Siemonsma and Hamon (2004) explained that local and introduced cultivars of *Ab. caillei* may flower within 50–110 days after sowing in the dry season (sowing in October: days shortening) and within 65–270 days after sowing in the rainy season (sowing in March: days lengthening). The farmers' varieties performed better than selected aphid-resistant accessions, except in Foumbot where the yields of Bangourain were lower than most accessions. Thus, the local materials or varieties were more adapted. Gombo caféier yielded better only in Foumbot, confirming that it is adapted to the Western highland. This is explained by the fact that the climate in the western highlands is cooler and the crop cycle is longer. Varieties such as Gombo caféier, Gombo paysan, Bangourain and VI060794 that are *A. caillei* have longer crop cycles (Kumar *et al.,* 2010). Hence, most local varieties performed better in their respective locations where they are cultivated by the farmer in terms of pod length and width and plant height.

CONCLUSIONS, RECOMMENDATIONS AND PERSPECTIVES

Conclusions

A. gossypii population attacking okra in Cameroon, India and Taiwan are not genetically different. Hence, okra accession(s) with good aphid resistance in Taiwan may react the same way in Cameroon, unless environmental factors alter the resistance reactions.

Out of 9 accessions (VI051114, VI036213, VI033805, VI041210, VI060818, VI039614, VI033824, VI060794 and VI060688) identified for management of *A. gossypii*, VI041210 in addition to VI057245 and Gombo caféier were the most resistant, while VI060794 and Kirikou were the most susceptible.

Leaf trichome was found to be the most significant biophysical bases of okra resistance to aphid, especially in the top stratum of the plant. Total leaf nitrogen plays a significant role in the susceptibility of okra accessions to aphids both constitutively and non-constitutively (aphid-induced plant resistance). The amount of total phenols and tannins in okra leaves may change following attack. Changes in total tannins could contribute to the resistance of okra accession to aphids. However, all the plant metabolites may play a role in the resistance or susceptibility of okra to aphids but varies among okra accessions.

The resistance category of okra to *A. gossypii* could be antibiosis or tolerance but not antixenosis (no none-preference). There was lower intrinsic rate of natural increase in all selected accessions, long developmental time in VI041210 and higher nymph mortality in most selected accessions; the yield of resistant accessions was lower than that of susceptible accession. Thus, aphid infestation did not affect the yield of the susceptible okra accessions. VI060794 was the highest yielding among the accessions, and which was moderately resistant during the first season; but was the most yielding in all ecozones in Cameroon and with some acceptable level of resistance (resistant during one of the seasons in each location). VI060794 was the only *Abelmouschus caillei* among the selected nine.

The level of resistance varied between accessions, location and time. VI036213 was the most resistant to aphids in the western highland; VI039614 was the most resistant in the monomodal humid rain forest of Buea in the South-West Region, VI060794 in the bimodal humid rain forest of Evodoula in the Center Region and Gombo cafiére, VI060818 and VI041210 in the soudano-sahelian region in Maroua, Far North Region. In addition to these accessions, we can add VI060794 and VI060818 in Buea, VI051115 and VI057245 in Evodoula, VI057245 in Foumbot for temporal stability in their respective locations. This temperal stability of resistance

permits these accessions to be cultivated at any time in their respective locations. None of the accessions showed spatial stability and may not be cultivated in all locations except for VI060794 that was actually resistant at least during one season in each location. Tolerance of VI060794 was the best across locations and with higher yield than the other selected accessions.

Recommendations

VI060794 should be promoted in breeding programs because of its moderate resistance and high yield, while paying attention to pod characteristics and mucilage. The breeders should consider yields of host plants when identifying okra germplasm resistant to *A. gossypii* since tolerance is the most important resistance category found in okra.

The susceptible accessions including Kirikou and VI060794 can be used as potential sources of tolerant traits. VI041210, VI051114, VI033824, VI057245 and VI036213 can be recommended for leaf trichomes, VI051114 and VI036213 for fruit size, VI041210, VI060794 and Gombo caféier for plant vigour. The various horticultural traits can be introduced in okra breeding programs for aphid resistance and productivity of okra.

In addition to VI060794, which is suitable for cultivation in all agro-ecological zones in Cameroon, VI036213 is suitable for resistance to aphids in the western highland, VI039614 in the monomodal humid rain forest, VI051114 for the bimodal humid rain forest and VI060818 and VI041210 in the soudano-sahelian zone of Cameroon.

Crop duration thus shows enormous variation depending on cultivar, locality and season. In *Ab. caillei* (West African okra), it varies from 4 months to well over 12 months. Comparing cultivars of similar earliness, it is striking that West African okra has a considerably longer productive period than cultivars of *Ab. esculentus* (common okra). Thus, Gombo cafeier and VI060794 can be recommended for home gardening since they have longer productive periods. Common okra such as VI041210 and VI057245 with shorter crop duration on the other hand can be recommended for market gardening.

Perspectives

An earlier study identified two biotypes (melon and cotton) of *A. gossypii*. Although these biotypes are morphologically indistinguishable, they have distinct host ranges. Studies have shown some differences in host preference and feeding behaviour between the melon and cotton

biotypes. Crop resistance to *A. gossypii* also has been shown to be biotype-specific. Efforts need to be made to identify which of the two biotypes is found on okra.

This study reveals spatial distribution of aphids on okra. The density of leaf trichomes also varies with plant strata. It could therefore be interesting to study the plant chemistry and investigate the effect of antibiosis and antixenosis according to plant stratum.

Mucilage is one of the major characteristics appreciated in okra by consumers who like sliminess. This has not been investigated and should be considered in subsequent studies and breeding depending on the target consumer.

More trials should be conducted in multilocations to increase the number of years of testing to confirm the yield performance and resistance under the different Cameroon agroecological zones.

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APPENDICES

Appendix 1: preliminary screening during spring (March to May) 2011 in Taiwan

Accessions	AUIPC (aphids per leaf)	VI056450	26.25 MR	VI055119	31.15 MS
VI051114	22.05 R	VI050957	26.25 MR	VI037994	31.15 MS
VI050958	22.05 R	VI055219	26.25 MR	VI041461	31.50 MS
VI059164	22.75 R	VI046554	26.60 MR	VI036203	31.50 MS
VI036213	23.45 R	VI054562	26.60 MR	VI040649	31.85 MS
VI046559	23.45 R	VI055996	26.60 MR	VI055421	31.85 MS
VI050960	23.80 R	VI039622	26.95 MR	VI046537	31.94 MS
VI033805	23.80 R	VI036212	26.95 MR	VI041462	32.55 MS
VI056457	23.80 R	VI050959	27.65 MR	VI055018	32.55 MS
VI033810	24.15 MR	VI055110	27.65 MR	VI056069	32.67 MS
VI047672	24.15 MR	VI049954	27.65 MR	VI033824	33.25 MS
VI048154	24.15 MR	VI055220	28.00 MR	VI033791	33.60 MS
VI049961	24.15 MR	VI033781A	28.35 MR	VI041139	34.30 MS
VI055424	24.15 MR	VI056079	28.44 MR	VI033785	35.00 S
VI049632	24.50 MR	VI039651	28.70 MR	VI056401	35.35 S
VI054546	24.50 MR	VI041215	28.70 MR	VI044241	35.35 S
VI047751	24.50 MR	VI037997	28.70 MR	VI039652	36.05 S
VI056456	24.50 MR	VI037995	28.70 MR	VI047808	36.05 S
VI050150	24.85 MR	VI046563	28.70 MR	VI057249	36.05 S
VI056404	24.85 MR	VI056455	29.05 MR	VI036215	36.05 S
VI046562	25.20 MR	VI056448	29.05 MR	VI055884	36.75 S
VI044233	25.55 MR	VI033781B	29.40 MS	VI039643	37.45 S
VI036211	25.55 MR	VI041763	29.75 MS	VI044244	37.80 S
VI040865	25.55 MR	VI050549	29.75 MS	VI036201	38.50 S
VI046544	25.55 MR	VI055423	29.75 MS	VI055422	43.40 HS
VI046566	25.55 MR	VI056451	29.75 MS	VI056452	43.40 HS
VI040770	25.90 MR	VI050956	30.10 MS	VI057245	51.10 HS
VI048291	25.90 MR	VI046536	30.10 MS	Overall mean (m)	35.48
VI059165	25.90 MR	VI050170	30.92 MS	S.D.	4.52
VI046556	26.25 MR	VI039638	31.15 MS		
VI056449	26.25 MR	VI033775	31.15 MS		

HR = Highly resistant, R= Resistant, MR = Moderately resistant, MS = Moderately susceptible, S = Susceptible, HS = Highly susceptible, S.D. = Standard deviation

Appendix 2: preliminary screening in autumn (September to November) 2011 at Taiwan

Accession	AUIPC (aphids per leaf)	Resistance status
VI058525	0	MR
VI058519	0.7	MR
VI058521	0.7	MR
VI060313	0.7	MR
VI058502	8.4	MR
VI058502	6.65	MR
VI058503	7.39	MR
VI058504	4.2	MR
VI058505	9.45	MR
VI058507	6.3	MR
VI058508	4.55	MR
VI058509	6.65	MR
VI058511	5.25	MR
VI058512	1.4	MR
VI058513	4.2	MR
VI058514	3.85	MR
VI058515	5.25	MR
VI058516	6.3	MR
VI058517	5.95	MR
VI058518	2.1	MR
VI058520	1.4	MR
VI058522	2.8	MR
VI058523	2.63	MR
VI058524	8.75	MR
VI058526	7.7	MR
VI058527	1.75	MR
VI058528	7.35	MR
VI058529	6.65	MR
VI058530	4.9	MR
VI058538	1.4	MR
VI060131	5.25	MR
VI060132	6.65	MR
VI060133	9.45	MR
VI060206	4.55	MR
VI060314	7.35	MR
VI060315	6	MR
VI060316	1.4	MR
VI060317	5.95	MR
VI051039	7.39	MR
VI051042	3.5	MR
VI051047	9.1	MR
VI051062	4.5	MR
VI041139	7	MR
VI041177	4.55	MR
VI037992	7.35	MR
VI037991	5.6	MR
VI037999	8.75	MR
VI033777	6.22	MR
VI033778	5.44	MR
VI033779	2.72	MR
VI033780	2.14	MR
VI033782	5.95	MR
VI033788	3.5	MR
VI046539	2.8	MR
VI033773	22.4	MS
VI054565	23.8	MS
VI054566	16.8	MS
VI058501	10.15	MS
VI058506	12.06	MS
VI058536	10.85	MS
VI051048	10.15	MS
VI046536	12.6	MS
VI033786	30.45	S
VI058500	25.28	S
VI058533	26.6	S
VI033784	54.6	HS
VI033789	92.12	HS
VI033824	61.85	HS
Overall mean	09.97	
S.D.	14.69	

HR = Highly resistant, R= Resistant, MR = Moderately resistant, MS = Moderately susceptible, S = Susceptible, HS = Highly susceptible, S.D. = Standard deviation

Appendix 3: preliminary screening in Cameroon (November 2011 to February 2012)

Accession	AUIPC (aphids per leaf)	VI060863	207.0 MR	VI060744	306.4 MR	VI060724	426.3 MS	VI060684	574.5 MS
VI060809	26.4 R	VI060683	210.0 MR	VI060697	309.4 MR	VI060842	431.0 MS	VI060754	579.4 MS
VI060810	30.3 R	CLEMSON SPINELESS	235.4 MR	VI060868	310.6 MR	VI060726	431.9 MS	VI060767	632.6 MS
VI060786	47.4 R	VI060866	238.2 MR	VI060706	312.9 MR	VI060848	433.1 MS	VI060824	643.5 MS
VI060702	86.6 R	VI060782	247.8 MR	VI060766	316.2 MR	VI060735	435.9 MS	VI060798	658.0 S
VI060787	95.4 R	VI060800	249.7 MR	VI060678	324.1 MR	VI060746	444.5 MS	VI060772	661.3 S
VI060740	102.7 R	VI060847	252.2 MR	VI060861	333.0 MR	VI060771	449.9 MS	VI060762	724.5 S
EVODOULA	104.5 R	VI060695	252.5 MR	VI060780	333.7 MR	VI060799	459.7 MS	VI060745	763.0 S
VI060704	105.2 R	VI060723	255.3 MR	VI060835	336.9 MR	GOMBO PAYSAN	460.3 MS	VI060855	820.2 S
VI060784	105.2 R	VI060808	255.3 MR	VI060689	340.9 MR	VI060715	462.7 MS	VI060743	825.1 S
VI060858	111.1 R	VI060789	259.5 MR	VI060693	340.9 MR	VI060821	466.0 MS	VI060862	889.2 S
Gombo caféier	118.3 MR	VI060845	261.1 MR	VI060805	345.3 MR	VI060713	469.2 MS	VI060833	939.2 HS
VI060774	122.7 MR	VI060691	270.7 MR	VI060857	345.3 MR	VI060870	469.2 MS	VI060852	989.8 HS
VI060823	131.1 MR	VI060728	273.7 MR	VI060831	349.8 MR	VI060707	487.0 MS	VI060869	1099.5 HS
VI060687	134.2 MR	VI060725	277.4 MR	VI060686	350.9 MR	VI060758	490.9 MS	VI060753	1184.6 HS
VI060785	137.4 MR	VI060690	277.7 MR	VI060682	357.7 MR	VI060763	509.4 MS	VI060756	1348.7 HS
VI060677	158.9 MR	VI060807	283.5 MR	VI060722	359.6 MR	VI060860	523.6 MS	VI060820	1459.3 HS
VI060718	160.1 MR	VI060864	286.8 MR	VI060788	365.9 MR	VI060832	532.9 MS	Overall mean	379.9
VI060739	163.8 MR	VI060856	287.7 MR	VI060708	367.5 MR	VI060872	540.4 MS		
VI060705	166.1 MR	VI060679	292.1 MR	VI060865	369.6 MR	VI060719	542.3 MS	Standard deviation	264.9
VI060727	169.4 MR	VI060710	295.2 MR	VI060717	372.9 MR	VI060676	544.8 MS		
VI060844	191.3 MR	VI060846	296.1 MR	VI060859	374.0 MR	VI060871	553.2 MS		
VI060851	199.7 MR	VI060760	300.5 MR	VI060720	394.1 MS	VI060854	568.6 MS		
VI060703	205.6 MR	VI060688	304.3 MR	VI060818	397.6 MS	VI060721	572.8 MS		

HR = Highly resistant, R= Resistant, MR = Moderately resistant, MS = Moderately susceptible, S = Susceptible, HS = Highly susceptible.

Appendix 4: preliminary screening in Spring 2012 (March to July) in Taiwan

Accession	AUIPC (aphids per leaf)	VI041444	2.5 MR	VI033789	17.5 MS
VI039617	24.5 MS	VI041450	0.7 MR	VI033796	*0 MR*
VI039639	11.6 MS	VI041451	0 MR	VI033800	0.7 MR
VI039646	13.7 MS	VI041457	0 MR	VI033804	4.5 MS
VI040634	4.2 MS	VI041463	0.7 MR	VI033812	2.5 MR
VI040643	13.7 MS	VI041464	0 MR	VI033815	4.2 MS
VI040660	17.7 MS	VI041465	1.8 MR	VI033818	2.1 MR
VI040681	0 MR	VI041467	7 MS	VI033819	3.2 MR
VI040986	3.5 MR	VI041470	1.6 MR	VI033822	0.4 MR
VI041207	4.9 MS	VI041472	0 MR	VI033824	*0 MR*
VI041213	3.5 MR	VI041682	1.4 MR	VI036207	0.7 MR
VI041214	1.4 MR	VI041716	2.1 MR	VI036210	0 MR
VI041216	1.4 MR	VI041654	0.8 MR	VI041231	3.3 MR
VI041217	0.7 MR	VI041655	3.9 MR	VI046534	6.3 MS
VI041218	0 MR	VI043568	1.4 MR	VI046535	6.2 MS
VI041220	0.7 MR	VI044234	0.4 MR	VI046537	*0 MR*
VI041222	0 MR	VI044236	4.2 MS	VI046539	0 MR
VI041227	8.8 MS	VI044237	0.7 MR	VI046540	2.5 MR
VI041228	4.9 MS	VI044239	*0 MR*	VI046541	2.0 MR
VI041229	2.8 MR	VI044242	2.8 MR	VI046547	0 MR
VI041230	2.5 MR	VI044495	1.8 MR	VI046549	0 MR
VI041232	5.4 MS	VI044496	3.9 MR	VI046550	0 MR
VI041237	0 MR	VI045117	10.2 MS	VI046553	0.8 MR
VI041240	0 MR	VI037992	28.7 MS	VI046557	0 MR
VI041244	0 MR	VI037991	9.8 MS	VI046558	5.6 MS
VI041250	9.5 MS	VI037999	9.8 MS	VI046564	1.4 MR
VI041253	1.4 MR	VI033777	0 MR	VI046567	0.7 MR
VI041259	2.8 MR	VI033778	20.0 MS	VI046568	0 MR
VI039810	3.1 MR	VI033779	13.7 MS	VI047712	8.8 MS
VI039823	0.4 MR	VI033780	16.5 MS	VI048221	8.8 MS
VI039825	1.9 MR	VI033782	9.8 MS	VI049957	4.2 MS
VI039835	1.4 MR	VI033783	1.8 MR	Overall mean	4.1
VI039836	1.8 MR	VI033788	1.8 MR	S.D.	41.97

HR = Highly resistant, R= Resistant, MR = Moderately resistant, MS = Moderately susceptible, S = Susceptible, HS = Highly susceptible, S.D. = Standard deviation

Appendix 5: preliminary screening in Autumn 2012 (September to November), Taiwan

Accession	AUIPC (Aphids per leaf)	Resistance status	VI060805	43.1	MR
VI033772	45.5	MR	VI060807	43.1	MR
VI033797	60.6	MS	VI060808	43.1	MR
VI033808	61.6	MS	VI060809	45.9	MR
VI033809	65.5	S	VI060817	37.8	R
VI033814	58.1	MS	VI060818	34.3	R
VI033823	59.5	MS	VI060819	55.7	MS
VI037993	52.2	MS	VI060820	52.5	MS
VI038288	60.6	MS	VI060821	49.0	MR
VI039614	36.4	R	VI060822	46.6	MR
VI039618	41.3	MR	VI060823	49.7	MR
VI039621	48.0	MR	VI060824	68.6	S
VI056406	51.1	MR	VI060827	61.3	MS
VI060676	53.9	MS	VI060828	56.4	MS
VI060677	47.6	MR	VI060829	64.8	S
VI060678	56.0	MS	VI060830	74.2	S
VI060679	59.9	MS	VI060831	41.7	MR
VI060680	46.9	MR	VI060832	55.0	MS
VI060681	58.1	MS	VI060833	68.6	S
VI060682	54.1	MS	VI060865	73.2	S
VI060683	72.5	S	VI060866	35.4	R
VI060684	44.1	MR	VI060867	51.8	MS
VI060685	42.7	MR	VI060868	52.9	MS
VI060686	46.6	MR	VI060869	85.2	HS
VI060687	51.5	MS	VI060872	41.0	MR
VI060688	30.8	R	VI060873	44.5	MR
VI060790	51.5	MS	VI060874	66.9	S
VI060792	50.4	MR	VI041209	75.1	HS
VI060793	46.6	MR	VI041210	30.5	R
VI060794	37.8	R	VI047660	43.4	MR
VI060802	45.9	MR	VI049964	34.3	R
VI060803	41.0	MR	VI060703	48.0	MR
VI060804	40.3	MR	Overall mean	51.4	
			S.D.	11.66	

HR = Highly resistant, R= Resistant, MR = Moderately resistant, MS = Moderately susceptible, S = Susceptible, HS = Highly susceptible, S.D. = Standard deviation

Appendix 6: mean infestation intensity of different okra varieties by the different species of the entomofauna during preliminary screening from November 2011 to February 2012 in Nkolbisson, Yaoundé

Accession	Aphidiidae		Chrysomelidae	Aleyrodidae	Noctuidae			Pyrrhocoridae	Clavicipitaceae	Coccinellidae	Syrphidae	Coccoidea
	Aphid	Aphid parasitiods	Leaf Beetles	White fly	Army worm	Looper	Leaf folder	Cotton stainer	Aphid parasitic fungi	Lady beetle	Syrphid	Mealybug
VI060820	62.3	0.1	0.3	1.1	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.3
VI060869	40.9	1.1	0.1	4.2	0.0	0.0	0.0	0.0	0.5	0.1	0.1	0.1
VI060756	40.8	0.0	0.2	5.7	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1
VI060726	38.8	0.3	0.6	17.6	0.0	0.0	0.1	0.0	0.2	0.0	0.1	0.1
VI060753	37.7	0.0	0.1	0.2	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
VI060852	31.8	0.0	0.1	2.9	0.0	0.0	0.0	0.0	18.8	0.0	0.2	0.1
VI060707	30.1	0.5	0.9	5.6	0.0	0.0	0.0	0.0	1.1	0.0	0.1	0.0
VI060868	30.0	0.1	0.3	3.2	0.0	0.0	0.0	0.0	0.0	0.1	0.3	0.0
VI060798	29.1	0.0	0.2	1.9	0.0	0.0	0.0	0.0	1.2	0.1	0.0	0.2
VI060725	28.9	0.2	1.6	17.5	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1
VI060855	28.7	0.0	0.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0
VI060833	28.2	0.2	0.1	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2
VI060728	28.0	0.1	0.8	14.4	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.1
VI060862	27.1	0.1	0.8	6.3	1.2	0.1	0.0	0.0	0.6	0.1	0.1	0.2
VI060745	26.6	0.1	0.3	2.5	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1
VI060743	26.0	0.4	0.1	1.5	0.0	0.0	0.0	0.0	0.5	0.1	0.1	0.1
VI060772	26.0	0.2	0.2	2.5	0.0	0.0	0.0	0.0	0.9	0.1	0.1	0.0
VI060676	25.9	0.0	0.3	0.2	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1
VI060762	25.4	0.0	0.1	7.7	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1
VI060821	24.9	0.2	0.3	2.7	0.0	0.0	0.0	0.0	0.2	0.2	0.1	0.0
VI060684	23.7	0.0	0.1	0.6	0.0	0.0	0.0	0.0	0.8	0.0	0.1	0.0
VI060854	23.4	0.1	0.1	8.7	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1
VI060767	21.2	0.1	0.2	3.6	0.0	0.0	0.0	0.0	0.5	0.1	0.0	0.0
VI060754	20.0	0.1	0.2	5.4	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0

VI060799	19.9	0.1	0.3	3.0	0.0	0.0	0.0	0.0	1.9	0.1	0.0	0.0
VI060824	19.9	0.0	0.1	4.7	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
VI060718	18.9	0.1	0.3	3.4	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0
VI060719	18.5	0.6	0.5	3.5	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.1
Gombo Paysan	18.4	0.1	0.5	4.3	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
VI060870	18.3	0.0	0.3	12.5	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1
VI060760	18.2	0.0	0.3	3.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.1
VI060871	18.0	0.2	0.3	3.4	0.0	0.0	0.0	0.0	0.1	0.2	0.1	0.1
VI060735	17.1	0.4	0.3	1.9	0.0	0.1	0.0	0.0	0.4	0.1	0.1	0.1
VI060860	17.0	0.2	0.5	4.0	0.3	0.0	0.0	0.0	0.8	0.0	0.0	0.0
VI060713	17.0	0.2	0.9	4.8	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0
VI060758	16.7	0.2	0.3	10.5	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0
VI060721	16.5	0.5	0.5	7.2	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1
VI060771	16.4	0.3	0.4	5.4	0.0	0.0	0.1	0.0	0.8	0.0	0.1	0.1
VI060746	16.4	0.0	0.2	1.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1
VI060682	16.4	0.1	0.1	0.4	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
VI060872	16.4	0.1	0.2	5.9	0.0	0.0	0.0	0.0	0.0	0.3	0.1	0.1
VI060832	16.3	0.4	0.2	0.9	0.0	0.0	0.0	0.0	0.2	0.0	0.1	0.2
VI060763	16.3	0.2	0.2	3.8	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.0
VI060744	16.2	0.0	0.0	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
VI060788	15.3	0.1	0.3	1.8	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.3
VI060717	15.2	0.2	0.3	4.2	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1
VI060842	15.0	0.4	0.3	5.4	0.0	0.0	0.2	0.0	0.8	0.0	0.1	0.3
VI060859	14.4	0.0	0.3	1.0	0.6	0.1	0.0	0.0	0.5	0.0	0.0	0.1
VI060722	14.0	0.3	0.6	10.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
VI060724	13.6	0.1	0.5	9.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
VI060848	13.6	0.0	0.2	3.2	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.1
VI060678	13.4	0.2	0.2	0.2	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.0
VI060706	13.4	0.5	0.4	3.6	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.1
VI060715	13.3	0.1	0.3	4.6	0.0	0.1	0.0	0.0	0.7	0.0	0.0	0.0
VI060831	12.9	0.1	0.4	2.5	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1
VI060766	12.8	0.2	0.1	2.6	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.1
VI060835	12.7	0.1	0.1	1.6	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.2
VI060818	12.5	0.0	0.4	2.1	0.0	0.0	0.1	0.0	0.0	0.7	0.0	0.0
VI060865	12.4	0.1	0.3	2.0	0.1	0.0	0.0	0.0	0.4	0.1	0.1	0.1
VI060710	12.2	0.8	0.8	8.9	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0
VI060784	11.9	0.0	0.4	1.8	0.0	0.0	0.0	0.0	3.6	0.0	0.0	0.1
VI060856	11.9	0.0	0.1	5.2	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0

VI060693	11.8	0.1	0.3	5.0	0.0	0.3	0.0	0.0	0.7	0.1	0.0	0.1
VI060689	11.6	0.1	0.1	1.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0
VI060686	11.4	0.3	0.4	0.8	0.0	0.0	0.0	0.0	0.6	0.1	0.0	0.0
VI060720	11.2	0.2	0.4	4.8	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.1
VI060800	11.2	0.4	0.3	2.5	0.0	0.0	0.0	0.0	0.7	0.1	0.0	0.1
VI060688	11.0	0.2	0.3	0.4	0.0	0.0	0.0	0.0	2.7	0.0	0.0	0.2
VI060805	11.0	0.1	0.5	2.5	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.1
VI060780	10.9	0.1	0.5	1.0	0.0	0.0	0.0	0.0	0.3	0.1	0.1	0.1
VI060774	10.9	0.1	0.5	3.4	0.0	0.0	0.0	0.0	0.4	0.0	0.1	0.1
VI060857	10.8	0.1	0.2	6.4	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1
VI060786	10.7	1.2	0.2	2.6	0.0	0.0	0.0	0.0	5.9	0.1	0.0	0.1
VI060861	10.6	0.1	0.2	3.4	0.6	0.0	0.0	0.0	0.9	0.1	0.1	0.0
VI060690	10.6	0.2	0.2	2.6	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.1
VI060697	10.5	0.1	0.2	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
VI060866	10.2	0.3	0.4	5.1	0.0	0.0	0.0	0.0	0.2	0.0	0.1	0.1
VI060679	9.9	0.2	0.2	0.4	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.1
VI060703	9.9	0.1	0.2	0.5	0.0	0.0	0.0	0.0	0.9	0.0	0.1	0.1
VI060687	9.6	0.0	0.4	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.1
VI060808	9.5	0.1	0.4	3.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.3
Clemson Spineless	9.4	0.1	0.2	4.7	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1
VI060864	9.4	0.1	0.4	4.0	0.5	0.0	0.0	0.1	0.2	0.0	0.0	0.2
VI060807	9.3	0.1	0.3	3.4	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.2
VI060695	9.1	0.1	0.3	1.5	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.1
VI060846	9.1	0.0	0.2	4.3	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0
VI060691	9.0	0.1	0.4	2.7	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0
VI060845	9.0	0.2	0.3	5.0	0.0	0.0	0.0	0.0	0.2	0.0	0.1	0.1
VI060858	8.8	0.0	0.1	7.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.3
VI060847	8.6	0.0	0.1	3.4	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.2
VI060789	8.4	0.1	0.1	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
VI060702	8.3	0.3	0.1	0.3	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.1

VI060704	8.3	0.0	0.5	3.6	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0
VI060863	8.1	0.1	0.4	8.2	0.4	0.0	0.0	0.0	0.1	0.0	0.0	0.1
VI060708	8.1	0.1	0.6	5.4	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
VI060782	8.1	0.1	0.9	1.9	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.1
VI060683	7.7	0.0	0.1	1.1	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.1
VI060727	7.3	0.1	0.7	9.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
VI060844	6.9	0.2	0.2	3.1	0.0	0.0	0.0	0.0	0.6	0.0	0.1	0.2
VI060851	6.4	0.1	0.2	5.1	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.0
VI060723	6.4	0.3	1.1	11.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
VI060810	6.3	0.0	0.2	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
VI060705	6.1	0.0	0.4	4.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
VI060785	5.8	0.2	0.2	2.3	0.0	0.0	0.0	0.0	0.2	0.0	0.1	0.1
VI060677	5.6	0.1	0.3	0.6	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
VI060739	4.9	0.3	0.2	6.6	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
Gombo caféier	4.8	0.0	0.4	17.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
VI060823	4.1	0.2	0.2	4.8	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0
VI060787	3.6	0.1	0.7	4.2	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0
VI060740	3.5	0.1	0.2	2.3	0.0	0.1	0.0	0.0	0.4	0.0	0.0	0.1
Evodoula	3.3	0.0	0.3	9.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
VI060809	2.1	0.1	0.3	6.3	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0
Correlation Coefficient for relationship between aphids and the other insects observed			*0.01*	*0.07*	*0.03*	*-0*	*-0.1*	*-0*	*0.13*	*0.23*	*0.48*	*0.14*

Appendex 7: mean infestation of different okra varieties by the different species of the entomofauna per leaf during the advanced replicated trial from March to July 2012 at Nkolbisson in Yaoundé

Accessions	Aphidiidae		Chrysomelidae	Aleyrodidae	Noctuidae		Pyrrhocoridae	Coccinellidae	Syrphidae	Dermaptera	Coccoidea
	Aphid	Aphid parasitoids	Leaf beetle	White fly adults	Looper	Leaf folder	Cotton stainer	Ladybird beetle	Syrphids	Earwig	Mealybug
Evodoula	7.9	0.00	1.0abcde	1.6	0.02	0.004	0.10	0.031	0.04	0.02	0.03
VI033805	1.9	0.00	0.6 de	0.7	0.00	0.00	0.00	0.004	0.00	0.03	0.004
TOT3145	3.6	0.00	0.5 e	0.5	0.00	0.00	0.01	0.004	0.02	0.01	0.00
TOT3879	4.2	0.00	0.7 cde	0.8	0.01	0.00	0.04	0.006	0.01	0.003	0.01
TOT6445	8.9	0.00	1.4abc	1.5	0.03	0.01	0.02	0.022	0.01	0.02	0.03
TOT6447	8.8	0.00	1.1abcde	0.9	0.01	0.04	0.06	0.011	0.05	0.02	0.02
TOT6599	3.4	0.00	1.1abcde	1.7	0.00	0.00	0.05	0.011	0.02	0.01	0.02
TOT7966	4.0	0.00	0.6 e	0.8	0.02	0.00	0.10	0.007	0.003	0.01	0.01
TOT8656	6.5	0.003	1.5ab	1.7	0.02	0.004	0.03	0.000	0.04	0.05	0.03
VI058519	5.3	0.00	0.8 bcde	1.4	0.02	0.00	0.01	0.003	0.02	0.03	0.003
VI058521	9.4	0.00	1.5ab	2.2	0.02	0.01	0.13	0.013	0.05	0.03	0.01
VI058525	8.9	0.00	1.4abcd	2.6	0.02	0.00	0.17	0.038	0.08	0.03	0.40
VI060313	12.5	0.00	1.8a	1.9	0.04	0.01	0.02	0.029	0.06	0.01	0.03
VI060740	8.5	0.00	1.0abcde	1.3	0.02	0.004	0.05	0.040	0.00	0.00	0.05
VI060784	7.2	0.004	1.4abc	1.0	0.02	0.00	0.02	0.018	0.04	0.01	0.01
VI060787	6.5	0.00	1.4abc	1.1	0.01	0.00	0.07	0.034	0.04	0.01	0.04
VI060809	5.2	0.00	1.4abcd	1.9	0.04	0.02	0.04	0.016	0.02	0.04	0.004
VI060810	7.7	0.00	1.2abcde	0.6	0.04	0.00	0.09	0.031	0.04	0.02	0.03
VI060858	5.4	0.00	1.6ab	1.2	0.02	0.00	0.02	0.004	0.00	0.03	0.004
$F_{18, 38}$ Value	1.32	0.95	2.67	1.15	1.12	1.22	1.65	1.18	1.62	0.76	1.01
Pr > F	0.2325	0.5352	0.0054	0.345	0.3749	0.293	0.0959	0.3228	0.1046	0.7306	0.4750

Mean values with different letters in a column are significantly different at $P < 0.05$.

Appendex 8: mean infestation of different okra varieties by the different species of the entomofauna per leaf during the advanced replicated trial from March to June 2013 at Nkolbisson in Yaoundé

Insect farmily	Insect common name	VI039614	VI041210	VI060688	VI060794	VI060817	VI060818	VI060866	$F_{6, 14}$ palue	*P-value*
Aphidiidae	Aphids	16.3ab	10.7b	12.6b	10.5b	24.5a	12.4b	16.8ab	*8.41*	*0.0005*
	Aphids parasitoids	0.00	0.00	0.00	0.004	0.00	0.00	0.00	*1.00*	*0.4628*
Chrysomelidae	Leaf beetle	1.3b	1.2b	1.6b	1.6b	2.9a	1.4b	1.5b	*5.22*	*0.0051*
Aleyrodidae	White fly	0.2e	0.7bc	0.3e	1.1ab	1.5a	0.3de	0.6cd	*29.76*	*<0.0001*
Clavicipitaceae	Aphid parasitic fungi	0.007	0.00	0.00	0.00	0.00	0.00	0.007	*0.85*	*0.5514*
Coccinellidae	Ladybird beetle	0.004	0.011	0.018	0.029	0.026	0.013	0.033	*0.53*	*0.7792*
Coccoidea	Mealybug	0.1b	0.1ab	0.1b	0.1ab	0.3a	0.1b	0.2ab	*3.84*	*0.0179*
Formicidae	Ants	0.28	0.34	0.08	0.21	0.76	0.11	0.40	*2.00*	*0.1336*

Mean values with different letters in a column are significantly different at $P < 0.05$.