Incidence, Distribution and Characteristics of Major Tomato Leaf Curl and Mosaic Virus Diseases in Uganda

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Thesis submitted in fulfilment of requirements for the degree of Doctor (PhD) in Applied Biological Sciences

Voorkomen, verspreiding en eigenschappen van de voornaamste Tomato Leaf Curl mozaïekvirusziekten in Uganda.

Illustrations:

Front cover: Left-top is an *Ocimum basilica* samples with yellow mosaic symptoms and infected with ChiVMV; mid-left are whiteflies (*B.tabaci*), the vector of tomato leaf curl viruses; bottom-left are tomato shoots showing leaf curl symptoms and marginal yellowing; top-right is one of our experimental tomato fields; bottom-right are quality fruits from a healthy tomato plant.
Back cover: Screen cages used to preserve our live tomato virus infected samples.

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CONTENTS

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To the MOVER, I surrender and mine is always HIS to move

SUMMARY

Tomato is economically important in Uganda whereby each of about 3 million households consume tomato in their sauce every meal. However, yields per hectare are still very low, i.e. 10 ton/ha (Ministry of Agriculture, Animal Industry and Fisheries (MAAIF) estimate for 1998 to 2000 unpublished). Low tomato yields in Uganda are mostly due to poor agronomic practices, lack of improved varieties, which are well suited for high yield and resistance to diseases, and also due to damage caused by pests and diseases (Varela, 1995; Hansen, 1990; Defrancq, 1989). As such, continued prevalence of viral diseases is considered to be the third major cause of low tomato productivity in Uganda after *Phytophthora infestans* blight and *Ralstonia solanacearum* bacterial wilt. As it is, this thesis presents the first research study on major leaf curl and mosaic virus diseases incidence, distribution, and characteristics in Uganda.

The study addressed these issues by firstly screening tomato viruses occurring in tomato fields and providing detailed information on genetic identity and vector relationship of one main virus disease, i.e. tomato yellow leaf curl virus (*sensu lato*) disease. To generate this information, three major activities were implemented. First, a survey was conducted in eight major tomato growing districts of Uganda, representing five agro-climatic zones. Virus-like symptoms were found on tomato plants in all eight districts surveyed. Two major categories of symptoms were encountered in surveyed tomato fields, i.e. leaf curl, and mosaic or mottling. Secondly, leaf samples taken from suspected virus-infected tomato plants were analysed to get unequivocal identification of viruses that occur in tomato in areas surveyed. A number of preliminary bioassays, such as indicator plant inoculation, and mechanical transmission as well as grafting, were conducted to confirm that leaf curl, mosaic and mottling symptoms were indeed due to virus infection. Serological and molecular virus identification tests were conducted using (1) antisera against ribonucleic acid (RNA) tomato viruses reported elsewhere in Africa; (2) general probes against deoxyribonucleic acid (DNA) geminiviruses; (3) specific probes against tomato yellow leaf curl viruses; and (4) general and specific primers in polymerase chain reaction (PCR). Viruses identified included *Tomato mosaic virus* (ToMV), *Cucumber*

m*osaic virus* (CMV), *Alfalfa mosaic virus* (AMV), *Pepper veinal mottle virus* (PVMV), *Potato virus Y* (PVY), *Potato virus X* (PVX), and *Tomato spotted wilt virus* (TSWV). Furthermore, three other viruses were identified, i.e. *Chili veinal mottle virus* (ChiVMV), *Tomato yellow leaf curl virus* (TYLCV) and *Tomato leaf curl virus* (ToLCV). ChiVMV (Brunt *et al*., 1990) is known to belong to genus *Potyvirus,* and causes moderate damage to tomato. The two tomato yellow leaf curl viruses, i.e. TYLCV-UG and ToLCV-UG, occur at high incidence in areas surveyed, and are causing important economic losses to tomato producers. TYLCV–UG and ToLCV-UG belong to genus *Begomovirus,* whose members are transmitted by a whitefly vector (*Bemisia tabaci*). TYLCV-UG is a strain of TYLCV-Israel (Is) (Russo *et al*., 1980; Czosnek *et al*., 1988). ToLCV-UG was identified as a begomovirus by using intergenic region and coat protein sequences (Brown, 1997). It had a DNA sequence homology of 89% < 90% with *African Tomato Leaf Curl Virus*-Tanzanian isolate (ATLCV-TZ; Chiang *et al*., 1996) for the 521 bp strand of the coat protein (CP) gene and part of the intergenic region (IR). Still with the same virus (ATLCV-TZ), but for the 482 bp sequence of the replication gene (Rep), ToLCV-UG had a low 4% < 90% homology. This begomovirus had fairly high homology (85% < 90%) with the *East African Cassava Mosaic Virus*-Malawi (EACMV-MW) isolate (Pita *et al*., 2001) for the CP gene. However, both CP gene homology percentages were considered to be below the required 90% nucleotide sequence identity (Padidam *et al*., 1995) for the two viruses to be similar. TYLCV-UG was closely related to TYLCV-EG (Nakhla *et al*., 1993) with a DNA sequence homology of 99% > 90% for the 277 bp of the intergenic region. TYLCV-EG is a strain of TYLCV-Is. As such, it was the first time the occurrence of these tomato viruses was established in Uganda. Based on its replication gene and coat protein sequences, ToLCV-UG is different from other tomato leaf curl viruses and is considered to be new in Uganda. Recent findings on tomato leaf curl viruses in Uganda, which were based on complete sequence comparisons (Shih, *et al*., 2006), confirmed our finding that tomato leaf curl virus (ToLCV-UG) is new.

Thirdly, field studies of the virus-vector relationship established that virus occurrence varied in space and time, and with management practices, crop development stage, and

weather conditions. A negative relationship ($R = -0.14$, $p = 0.04$) was established between number of plants infected with TYLCV (*sensu lato*) and percentage marketable tomato yield. On the other hand, *Bemisia tabaci*, the vector of TYLCV *(sensu lato)* showed a variable population, which depended on micro-climatic conditions in the agro-ecosystem, with high populations prevailing during the dry season and decreasing with the onset of rain, and in turn influenced tomato yellow leaf curl virus disease incidence. The more mature the tomato crop, the less it was infested with whiteflies ($R = -0.5$, $p < 0.0001$), for whiteflies prefer tender leaves, which are found on young tomato plants (Nono-Womdim *et al*., 1996). Therefore, variation in date of planting could be used in management of both whiteflies and TYLCV (*sensu lato*). Furthermore, an integrated package of uprooting TYLCV disease symptom bearing plants and application of the insecticide dimethoate was found to be the most effective of the six treatments applied in reducing whitefly populations and controlling TYLCV (*sensu lato*). Perring *et al*. (1999), while considering the effect of epidemiological factors and transmission of insect-vectored viruses on the effectiveness of chemical treatments, found that the best approach to vector and virus disease management was to use more than one control measure. Treatments applied during our study indicated that uprooting combined with application of dimethoate was the most effective control. Chan and Jeger (1994) reported that uprooting was more effective especially when plants are sparsely planted. Tomato is densely planted and canopies overlap. Even though, our finding indicated that at low disease incidence both chemical and uprooting were individually effective. Vaseline-smeared sticky traps made from locally available 5 litre yellow plastic jerry cans, were used to monitor infestation. They trapped an average of 100 whiteflies per $1m^2$, and had efficiency either of 483, 100 or 117 whiteflies per $1m^2$ for the first, second, and third planting experiment, respectively.

These results present a good starting point for tomato virus diseases diagnosis in Uganda; throw more light on the use of partial sequences to compare geminiviruses; and give sound tomato yellow leaf curl virus disease and vector management options.

Consequently, our findings concur with hypotheses formulated at the onset of the study, and the following is the summary of our key conclusions and recommendations:

1- Several viruses, including those already reported in East Africa, infect tomato in Uganda.

2- A number of viruses infect Ugandan tomato, and cause virus diseases, which are characterized by symptoms such as leaf curl, mosaic and mottling. These viral disease symptoms are not specific to a particular virus.

3- Viruses responsible for a number of leaf curl, mosaic and mottling virus symptoms observed on tomato were reported for the first time, in Uganda, i.e. RNA viruses ChiVMV, PVMV, AMV, CMV, TSWV, PVY and PVX, as well as begomoviruses, i.e. ToLCV-UG and TYLCV-UG. Basing on the genetic composition of the replication gene and coat protein gene, and to the best of our knowledge, ToLCV-UG is a new virus and reported for the first time in Uganda. Whiteflies were established to be vectors of identified begomoviruses. Whitefly adults are not easy to count because they fly away immediately when their resting ground is disturbed. A new sampling tool, i.e. the Kubwa sticky trap, for trapping adult whiteflies on individual tomato plants, and made from locally available plastic materials, would serve as new format of sticky traps to be used for whitefly monitoring before farmers decide to spray. This way, farmers would minimize amount of pesticides applied and leaking to the environment.

4- Combinations of two to five viruses exist in individual tomato plants. In one experiment, it was observed that PVMV alone caused no visible significant damage to the crop, but co-existence with ChiVMV in the same plant showed significantly severe and synergistic effects on tomato crop performance. These mixtures could be contributing to the severity of tomato virus diseases in Uganda.

5- Tomato yellow leaf curl viruses are amongst major viruses whose incidence and spread are influenced by presence of the whitefly vector (*Bemisia tabaci*) as well as weather conditions within the agro-ecosystem in Uganda.

6- We established that weeds act as alternative hosts to some tomato viruses identified, which lead to a clear understanding of the necessity for timely weeding of tomato fields as an option for virus disease management.

7- Majority of Ugandan tomato growers are smallholder farmers. They try to avoid risks related to farming by growing a multiplicity of crops, and this practice contributes to creating a complex nature of the agro-ecosystem favourable for both tomato viruses and their vectors.

8- It was found that whiteflies preferred young tomato plants to old plants. It is recommended that whiteflies be controlled at nursery stage before transplanting seedlings, before infection or when vector and disease levels are still very low.

9- Uprooting diseased plants and applying dimethoate was an effective control for whitefly vectors. Tomato growers are advised to apply this integrated management package for TYLCV (*sensu lato*) and whitefly vector control, especially if done during the dry season and the first part of the rainy season when whitefly populations are high. In this way, farmer expenditure on pesticides would be minimized and the amount of pesticides filtering through to the environment would be tremendously reduced, even though both economic analysis and evaluation of pesticide pollution were not conducted.

10- Having known major viruses infecting tomato, identified causal organisms of yellow leaf curl symptoms and their vector, and established appropriate integrated management practices, future research efforts should focus on the following;

- conduct a survey of tomato viruses to cover agro-climatic zones that were not surveyed during our study;
- investigate further the biodiversity, incidence, host range and mode of transmission of ChiVMV in Uganda;
- investigate the occurrence of TYLCV-UG and ToLCV-UG in other districts of Uganda; and study its biodiversity in relationship with whitefly vectors observed on tomato and on other plants within the tomato agro-ecosystem.
- evaluate tomato varieties, both transgenic and non-transgenic, for resistance to TYLCV-UG and ToLCV-UG.

SAMENVATTING

Tomaat is een economisch belangrijk gewas in Oeganda; het wordt er in ongeveer 3 miljoen huishoudens in elke maaltijd verwerkt en in bijna alle agro-ecologische klimaatszones gekweekt. Nochtans is de oogst per hectare erg laag (10 t/ha, schatting van het Ministerie voor Landbouw, Veeteelt en Visserij (MAAIF) voor 1998 tot 2000, ongepubliceerd). De lage oogst is vooral te wijten aan gebrekkige landbouwpraktijken; schade veroorzaakt door ziekten en plagen en een tekort aan variëteiten met een hoog opbrengstpotentieel die resistent zijn tegen deze ziekten en plagen (Varela, 1995; Hansen, 1990; Defrancq, 1989).

Virale ziekten worden beschouwd als de derde voornaamste oorzaak van de lage productiviteit van tomaat in Oeganda, na *Phytophthora infestans* en de bacteriële verwelkingziekte veroorzaakt door *Ralstonia solanacearum.* Huidig werk is het resultaat van het eerste onderzoek op het voorkomen, de verspreiding en de karakteristieken van tomatenkrulblad- en mozaïekvirussen in Oeganda.

Het eerste luik van het onderzoek spitste zich toe op de identificatie van tomatenvirussen die aangetroffen worden in de velden en op de verzameling van gedetailleerde informatie over de genetische identiteit en vector relaties van één van de voornaamste virale aandoeningen: het gele tomatenkrulbladvirus (*sensu lato*).

Deze informatie werd in drie stappen gegenereerd. Ten eerste werden symptomen die wijzen op de aanwezigheid van virussen in kaart gebracht in acht belangrijke tomaat producerende gebieden, verspreid over vijf agro-klimatologische zones. Symptomen die door virussen veroorzaakt kunnen worden, werden in de acht onderzochte districten waargenomen. De symptomen konden in drie klassen ingedeeld worden: bladkrul, mozaïek en vlekken.

In een tweede stap werden bladstalen die genomen waren op mogelijk geïnfecteerde tomatenplanten geanalyseerd om de virussen te identificeren. Een paar preliminaire tests, zoals indicatorplant inoculatie, mechanische transmissie en enten, werden uitgevoerd om

te bevestigen dat bladkrul, mozaïek en vlekken-symptomen effectief te wijten waren aan een virale infectie.

Serologische en moleculaire virus identificatie tests werden uitgevoerd met behulp van (1) antisera tegen ribonucleinezuur (RNA) tomatenvirussen die elders in Afrika gesignaleerd zijn; (2) algemene peilingen naar deoxyribonucleinezuur (DNA) geminivirussen; (3) specifieke testen naar gele tomaatbladkrulvirussen; en (4) algemene en specifieke *primers* in polymerase kettingreactie (PCR).

Onder de geïdentificeerde virussen bevonden zich de volgende: tomaatmozaïekvirus (ToMV), komkommermozaïekvirus (CMV), luzernemozaïekvirus (AMV), paprikanerfvlekkenvirus (PVMV), aardappelvirus Y (PVY), aardappelvirus X (PVX) en tomatenbronsvlekkenvirus (TSWV). Daarenboven werden nog drie andere virussen geïdentificeerd: het Spaanse peper nerfvlekkenvirus *Chili veinal mottle virus* (ChiVMV), het gele tomatenkrulbladvirus (TYLCV) en het tomatenkrulbladvirus (ToLCV). ChiVMV (Brunt *et al*., 1990) behoort tot het genus *Potyvirus*, en veroorzaakt slechts lichte schade aan tomaat.

De twee tomatenkrulbladvirussen, TYLCV-UG en ToLCV-UG zijn wijd verbreid in de onderzochte gebieden, en veroorzaken er belangrijke economische verliezen. TYLCV-Uganda (UG) en ToLCV-Uganda (UG) behoren tot het genus *Begomovirus,* wiens leden verspreid worden via een witte vlieg vector (*Besima tabaci*). TYLCV-UG is nauw verwant met TYLCV-Israël (Is) (Russo *et al*., 1980; Czosnek *et al*., 1988).

ToLCV-UG werd geïdentificeerd als begomovirus door het gebruik van intergenetische regio-en manteleiwit sequenties. Het heeft een DNA sequentiehomologie van 89% met het Afrikaanse tomatenkrulbladvirus – Tanzania isolaat (ATLCV-TZ; Chiang *et al*., 1996) voor het 521basenpaar (bp) streng van het manteleiwit gen (CP) en een deel van de tussengen regio (IR). Voor hetzelfde virus (ATLCV-TZ), had ToLCV-UG een lage 4% homologie met de 482 bp sequentie van het replicatie gen (Rep). Dit begomovirus had een redelijk grote homologie (85%) met het Oost Afrikaanse cassave mozaïekvirus – Malawi (EACMV-MW) isolaat (Pita *et al*., 2001) voor het manteleiwit gen.

Beide genhomologie percentages waren echter onder de vereiste 90 % nucleotide sequentie identiteit (Padidam *et al*., 1995) opdat de virussen als gelijksoortig beschouwd mogen worden. TYLCV-UG was nauw verwant met TYLCV-Egypte (EG) (Nakhla *et al*., 1993), met een DNA sequentie homologie van 99% voor het basenpaar 277 van de tussengen regio. TYLCV-EG is nauw verwant met TYLCV-Is. Dit is de eerste keer dat de aanwezigheid van dit virus aangetoond is in Oeganda.

Recente bevindingen over tomatenkrulbladvirussen, gebaseerd op de vergelijking van de volledige sequenties (Shih, *et al*., 2006), bevestigen dat het tomatenkrulbladvirus ToLCV-UG nieuw is.

In een derde fase werd de virus - vector relatie in veldomstandigheden onderzocht. Het voorkomen van de virussen varieert in tijd en ruimte en naargelang beheerspraktijken, het ontwikkelingsstadium van het gewas en de weersomstandigheden. Er werd een negatieve relatie ($R = -0.14$, $p = 0.04$) tussen het aantal planten aangetast door TYLCV (*sensu lato*) en het percentage vermarktbare tomatenoogst vastgesteld. Er werd waargenomen dat *Bemisia tabaci*, de vector van TYLCV (*sensu lato*) een variabele populatie had, afhankelijk van micro-klimatologische omstandigheden in het agro-ecosysteem, met hoge populatiedensiteit in het droog seizoen, die dan verminderde als het begon te regenen. Deze variatie beïnvloedde het voorkomen van het gele tomaatkrulbladvirus. Hoe rijper het gewas, hoe minder witte vlieg erop te vinden was ($R = -0.5$, $p < 0.0001$), aangezien witte vlieg een voorkeur heeft voor zachte bladeren, die voornamelijk terug te vinden zijn op jonge tomatenplanten (Nono-Womdim *et al*., 1996). Variatie in plantdatum zou dus een mogelijke beheersoptie zijn om zowel witte vlieg als TYLCV (*sensu lato*) te bestrijden.

Van zes uitgevoerde behandelingen bleek een integrale aanpak van uitgraven van planten die symptomen van aantasting door TYLCV vertonen en toepassing van dimethoaat insecticide de meest doeltreffende methode om TYLCV (*sensu lato*) te bestrijden.
Perring *et al.* (1999), die het effect van epidemiologische factoren en transmissie van virussen die door insecten verspreid worden op de doeltreffendheid van chemische

behandelingen bestudeerden, kwamen tot de conclusie dat de beste aanpak van virus- en vectorbestrijding een combinatie van beheersmaatregelen is. Chan and Jeger (1994) stellen dat wieden doeltreffender is, zeker waar planten ver uit elkaar staan. Tomaat wordt echter dicht geplant en het gebladerte overlapt. Resultaten van dit werk geven aan dat indien er slechts weinig planten aangetast zijn door de virussen, zowel chemische behandeling als uitgraven individueel doeltreffend zijn.

Om de aanwezigheid van witte vlieg op te volgen werden lokale gele plastic potten met een inhoud van 5 liter gebruikt die ingesmeerd waren met vaseline. Er werden gemiddeld 100 witte vliegen per m² gevangen; de efficiëntie van de vallen was respectievelijk 483, 100 en 117 per m² in de eerste, tweede en derde plantproef.

Deze resultaten zijn een goed startpunt voor de diagnose van virale aandoeningen op tomaat in Oeganda; ze belichten het gebruik van partiële sequenties om geminivirussen te vergelijken; en bieden valabele beheersopties in de bestrijding van het geel tomaatkrulbladvirus en zijn vectoren.

De resultaten van het onderzoek bevestigen de hypotheses die aanvankelijk geformuleerd waren. Hieronder het overzicht van onze voornaamste conclusies en aanbevelingen:

1. Verscheidene virussen, onder andere deze die reeds eerder vermeld waren in Oost Afrika, infecteren tomaat in Oeganda
2. Verschillende virussen die tomaat in Oeganda aantasten, veroorzaken ziekten die gekarakteriseerd worden door symptomen als bladkrul, mozaïek en vlekken. Deze symptomen zijn niet toe te schrijven aan een specifiek virus.
3. Virussen verantwoordelijk voor een aantal bladkrul, mozaïek en vlekken symptomen die op tomaat geobserveerd worden zijn voor het eerst geobserveerd in Oeganda, dit zijn de RNA-virussen ChiVMV, PVMV, AMV, CMV, TSWV, PVY en PVX, alsook de begomovirussen ToLCV-UG en TYLCV-UG. Voorzover ons bekend, is ToLCV-UG een nieuw virus dat bovendien voor het eerst werd waargenomen in Oeganda. De resultaten van

dit onderzoek geven ook aan dat witte vliegen de vectoren zijn van de geïdentificeerde begomovirussen. Volwassen witte vliegen zijn moeilijk te tellen aangezien ze onmiddellijk wegvliegen als hun omgeving verstoord wordt. De voor dit werk ontwikkelde Kubwa plakval, die dient om volwassen witte vliegen op individuele tomatenplanten te vangen en daarenboven gemaakt is uit lokaal beschikbaar materiaal, kan dienen als nieuw type val die gebruikt kan worden om de aanwezigheid van witte vlieg op te volgen voordat landbouwers besluiten om eventueel over te gaan op chemische bestrijding. Op deze manier zullen landbouwers de hoeveelheid gebruikte pesticiden reduceren tot een minimum, waardoor er ook minder schade aan de omgeving zal zijn.

4. In individuele tomatenplanten zijn vaak twee tot vijf virussen tegelijk aanwezig. In één experiment werd vastgesteld dat PVMV geïsoleerd geen significante schade toebracht aan het gewas, maar dat co-existentie met ChiVMV in dezelfde plant de plant wél ernstig beschadigde. De aanwezigheid van verschillende virussen in één plant dragen dus bij tot de omvang van het effect van virussen op de tomatenoogst in Oeganda.
5. Het gele tomatenkrulbladvirus is één van de voornaamste virussen wiens voorkomen en verspreiding beïnvloed worden door de witte vlieg vector (*Bemisa tabaci*) en door de weersomstandigheden in het agro-ecosysteem in Oeganda.
6. In dit werk werd vastgesteld dat onkruid de functie van gastheer voor bepaalde tomatenvirussen soms overneemt; het regelmatig wieden van tomatenvelden is dus een belangrijke optie voor de bestrijding van virale aandoeningen bij tomaat.
7. Het merendeel van de tomatenproducenten in Oeganda zijn kleinschalige landbouwers. Zij trachten risico's te beperken door verschillende gewassen te telen. Dit gebruik draagt echter bij tot de creatie van gunstige omstandigheden voor zowel de virussen die tomaat aantasten als hun vectoren.

8. Dit onderzoek wijst uit dat witte vliegen jonge tomatenplanten prefereren ten opzichte van oudere planten. Het wordt aanbevolen witte vlieg te bestrijden in de kwekerij, vooraleer de zaailingen verplant worden, voordat de planten geïnfecteerd zijn of als het aantal geïnfecteerde planten nog klein is.
9. Zieke planten uitgraven en dimethoaat toepassen is een doeltreffende manier om witte vlieg te bestrijden. Tomaatkwekers worden geadviseerd deze integrale aanpak te gebruiken in de strijd tegen TYLCV (*sensu lato*) en zijn vector witte vlieg; en voornamelijk tijdens het droog seizoen en het eerste deel van het regenseizoen, als witte vlieg een grote populatie densiteit heeft. Ondanks het feit dat noch een economische analyse noch een evaluatie van het effect van pesticiden op het milieu uitgevoerd werden binnen het kader van deze studie, kan besloten worden dat met deze aanpak het gebruik van pesticiden daalt; de uitgaven van de landbouwers voor pesticiden eveneens; en de hoeveelheid pesticiden die in het milieu terecht komt drastisch gereduceerd wordt.
10. De voornaamste virussen die tomaat aantasten zijn gekend; de organismen die gele tomaatkrulblad symptomen veroorzaken alsook hun vector zijn geïdentificeerd; en geschikte beheersmaatregelen om die te bestrijden, zijn bepaald. Verder onderzoek in de toekomst zou zich vooral moeten toespitsen op de volgende aspecten:
    a. in kaart brengen van de virussen die tomaat aantasten in de agro-klimatologische gebieden die niet in deze studie opgenomen waren;
    b. verder onderzoek op de biodiversiteit, het voorkomen, de waaier aan gastheersoorten en de transmissie van ChiVMV in Oeganda;
    c. onderzoek naar het voorkomen van TYLCV-UG en ToLCV-UG in andere districten van Oeganda, alsook naar de biodiversiteit van deze soorten en hun relatie met hun vector witte vlieg die op tomaat en andere planten in het agro-ecosysteem waargenomen zijn;
    d. tomaat variëteiten onderzoeken, zowel transgeen als niet transgeen, op weerstand tegen TYLCV-UG en ToLCV-UG.

LIST OF TABLES

LIST OF FIGURES

ACRONYMS AND ABBREVIATIONS

Abbreviation	Word in full
A	Adenine
AAP	Acquisition Access Point
AC	Coat protein
AMV	*Alfalfa mosaic virus*
ANOVA	Analysis of variation
ARP	Africa Regional Programme
ASL	Above Sea Level
ATLCV	*African tomato leaf curl virus*
ATLCV-TZ	*African tomato leaf curl virus*-Tanzania
Au	Australian strain
AVRDC	Asian Vegetable Research Institute
BGMV	*Bean golden mosaic virus*
BI	Bean integration
BID	Bean integration and Dimethoate
bp	Base pair
C	Cytosine: complementary sense polarity
c1-c6	Treatments 1-6
CGIAR	Consultative Group on International Agricultural Research
ChaMV-Ng	*Chayote mosaic virus* Nigeria strain
ChiVMV	*Chili veinal mottle virus*
CMGs	Cassava mosaic geminiviruses
CMI	Commonwealth Mycological Institute
CMV	*Cassava mosaic virus*
Cu	Cuba strain
CVMVmxd	*Chili veinal mottle virus* mixed sample
DAS	Double Antigen Sandwich
DAS-ELISA	Double Antigen Sandwich-Enzyme Linked Immunosorbent Assay
dATP	d-amino triphosphate
dCTP	d-cytosine triphosphate
df	Degrees of freedom
dGTP	d-guanosine 5'-triphosphate
DNA	Deoxyribonucleic acid
dNTP	d-Nitritriphosate
DR	Democratic Republic
dTTP	d-Thymine triphosphate
E	East
EACMV	*East African cassava mosaic virus*
EACMV-MW	*East African cassava mosaic virus*-Malawi
TYLCV-EG	Egyptian strain
EG	Egypt
ELISA	Enzyme Linked Immunosorbent Assay
f	Forward

Abbreviation	Word in full
FAO	Food and Agriculture Organization
FDP	Foundation for Development Programme
G	Guanosine, or Guanine
GTZ	German Agency for Technical Cooperation
IAP	Inoculation Access Point
ICTV	International Committee for Taxonomy of Viruses
IDPS	Internally Displaced People
IFAD	International Fund for Agricultural Development
IG	Iganga
IG 1	Iganga 1
IgM	Immuno-globulin M
TYLCV-IN	India strain
IR	Intergenic Region
ISO	Isolate
ISOPOT	Isolate in Pot
IUCN	International Union for Conservation of Nature
TYLCV-JR	Japanese strain
K	Kawanda
KA	Kasese
KDA	Kitgum Development Association
Ma	Malaysian strain
MAAIF	Ministry of Agriculture Animal Industries and Fisheries
MB	Mbale
MC	Monocrop
MD	Monocrop and Dimethoate
MP	Mpigi
MPz	Mpigi sample z
MR	Mbarara
Msc	Master of Science
MSE	Mean square error
MU	Mukono
MU	Monocrop and Uprooting
MUK	Makerere University
MW	Malawi
N	Nitrogen
n1-n6	Treatments 1-6
NAADS	National Agricultural Advisory and Development Services
NaOH	Sodium hydroxide
NARO	National Agricultural Research Organization
NP	Nepal strain
NUSAF	Northern Uganda Agricultural Fund
ORF	Open Reading Frame
OyVMV-PK	*Okra yellow vein mosaic virus*-Pakistan strain
p	Probability

Abbreviation	Word in full
PCR	Polymerase Chain Reaction
Ph	Philipines strain
PVMV	*Pepper veinal mottle virus*
PVX	*Potato viurs X*
PVY	*Potato virus Y*
r	Reverse
RA	Rakai
RL	Sample from Ranch on the Lake
RNA	Ribonucleic Acid
S	South
SA	South African strain
SADC	South African Development Cooperation
SD	Sudan
SR	Sardinia
T	Thymine
TAS	Triple-Antibody Sandwich
TAV	*Tomato aspermy virus*
TbLCZbwV	*Tobacco leaf curl Zimbabwe virus* strain
TE	(Tris-EDTA buffer)Tris-EthyleneDiamineTetraaceticAcid
TIC	*Tomato infectious chlorosis*
TICV	*Tomato infectious curl virus*
TLCV-SD	*Tomato leaf curl virus*-Sudanese strain
TMV	*Tobacco mosaic virus*
ToLCV	*Tomato leaf curl virus*
ToLCV-UG	*Tomato leaf curl virus*-Ugandan strain
ToLCV-UGf	*Tomato leaf curl virus*-Uganda forward (sequence)
ToLCV-UGr	*Tomato leaf curl virus*-Uganda reverse (sequence)
ToMV	*Tomato mosaic virus*
TSWV	*Tomato spotted wilt virus*
TW	Taiwan strain
TYLCV	*Tomato yellow leaf curl virus*
TYLCV-Is	*Tomato yellow leaf curl virus*-Israel strain
Tz	Tanzanian strain
UG	Uganda
UK	United Kingdom
US	United States
USA	United States of America
USDA	United States Development Agency
UV	Ultra Violet
UWA	Uganda Wild Life Association
V (1,2)	Viral sense polarity
W	West
WFP	World Food Programme

CHAPTER 1

GENERAL INTRODUCTION

This chapter aims at introducing our study of the incidence, distribution, and characteristics of tomato leaf curl and mosaic virus diseases in Uganda. It presents the research problem and set objectives.

1 CHAPTER 1

1.1 General Introduction

1.1.1 Tomato

The tomato (*Lycopersicon esculentum* Mill.) is a herbaceous fruiting plant. It originated in Latin America and has become one of the most widely grown vegetables with ability to survive in diverse environmental conditions (Rice *et al.,* 1987). Tomato fruit is considered to be fairly high in vitamins A and C (Table 1.1), of high cash value and with potential for value-added processing. Tomato was regarded as a top priority vegetable by scientists interviewed under the Technical Advisory Committee of the Consultative Group on International Agricultural Research (CGIAR) (FAO, 1990). Recently, there has been more emphasis on tomato production not only as source of vitamins, but also as a source of income and food security. Consequently, tomato is considered by the National Agricultural Research Organization (NARO) to be a top priority among other vegetables in Uganda (Valera, 1995; NARO, 1999). Tomato grows best in fertile, well-drained soils, with pH 6 and ambient temperatures of about 25 ^{0}C (Villareal, 1979; Rice *et al*., 1987). These conditions are common in East Africa, and Uganda in particular.

Table 1.1: Nutrient contents of vegetables per 100 g of edible parts (AVRDC, 2004 Training Notes)

Product	***Energy (Cal.)***	***Protein (g)***	***Calcium (mg)***	***Iron (mg)***	***Vitamin A (mg)***	***Vitamin C (mg)***
Lycopersicon esculentum	19	1.1	6	0.6	79	22
Capsicum annuum	23	1.1	10	0.8	253	90
Allium cepa	31	2.7	53	2.3	153	32
Cucumis melo	37	1	20	0.4	181	10
Brassica oleracea var. *capitata*	19	1.4	47	0.7	40	39
Phaseolus vulgaris	301	22.2	242	6.1	6	0
Capsicum frutescens	-[1]	-	20	2	77	120
B.oleracea var. *acephala*	-	-	220	0.8	29	81
Raphanus sativus	-	-	170	1.4	23	49
B.oleracea var. *gemmifera*	-	-	37	1	7.1	160
B.oleracea var. *botrytis*	-	-	38	1	8.1	120

[1] (-) information not known

1.1.1.1 Social-economic Importance of Tomato in Uganda

According to FAO (1990, 2003) reports, tomato is now the most important vegetable in the tropics. It is annually planted on almost 4 million ha worldwide. According to statistical production data, in Tanzania which is one of the countries neighbouring Uganda, tomato yields are estimated to be 10 to 14 t/ha (Nono-Womdim *et al.*, 1996), while in Sudan, tomato yields of about 20 t/ha have been reported (Yassin, 1989). In some eastern and southern Africa countries, tomato yields on smallholder farms do not exceed 20 t/ha. However, a smallholder commercial farmer is expected to get tomato yields of at least 100 t/ha (AVRDC, 1994). In Uganda, by 1990, most smallholder tomato producers were concentrated in the Lake Victoria basin region with about 150 to 500 hectares under tomato, and with an average annual production level of 10 t/ha (Ugandan Ministry of Agriculture Animal Industry and Fisheries (MAAIF) statistics, unpublished; Baliddawa, 1990). Since then, tomato is grown and consumed in every district of Uganda (Mukiibi, 2001; Mwaule, 1995). According to now available data, agricultural yields annually increase with 5% (MAAIF Planning Unit unpublished verbal communication). In the absence of concrete data on tomato production, and taking this growth in yield to be true also for the tomato crop, an annual production level of about 12.5 t/ha is expected by 2006, which is still very low.

1.1.1.2 Factors Limiting Tomato Yield

Low tomato yields are due to a number of factors. These include (1) lack of improved well-performing varieties; (2) poor fruit setting due to heavy rains and excessively high temperatures, which limit pollination, more specifically fecundation plus pollen viability; and (3) pests and diseases (Villareal, 1979; Lyons *et al.*, 1985; Ladipo, 1988). In eastern and southern Africa, arthropods, and fungal as well as bacterial diseases are considered to be the major constraints to tomato production. Viral diseases have been ranked as the third most important constraint among tomato diseases, basically because of absence of enough information on them (Varela, 1995).

In the case of Uganda poor tomato yield is attributed to three factors, (a) lack of improved varieties, which are well suited for high yield and resistance to diseases, (b) occurrence of pests and diseases, and (c) lack of knowledge on sustainable agronomic practices (Defrancq, 1989; Hansen, 1990; Mwaule, 1995). Tomato varieties Caribe, Magrobe, Pakmor and Tropic are reported to be acceptable to tomato farmers and consumers in Uganda, while tomato varieties VF 6203 and Peto-C-8100 159 with resistance to *Verticillium* spp. and *Fusarium* spp. are recommended for processing (Mwaule, 1995). Mwaule further confirmed the Asian Vegetable Research and Development Centre (AVRDC) tomato lines MT 40, 41, 55, 56 and 57 to be resistant to bacterial wilt (*Ralstonia solanacearum*). It is also reported that sustainable agronomic practices, such as plant spacing of 45 cm x 90 cm, mulching, staking and pruning to two leader vines, help to achieve better fruit quality and higher yields (Mwaule, 1995; Rice *et al*., 1987). Pests and diseases such as blight (*Phytophthora infestans* and *Alternaria solani*), bacterial wilt (*R. solanacearum*), root nematodes (*Meloidogyne* spp.), African bollworm (*Helicoverpa armigera*), thrips (*Thrips tabaci*), whitefly (*Bemisia tabaci*) and aphids (*Myzus persicae*) are reported to infect tomato (Defrancq, 1989; Mwaule, 1995). There are also a number of viral diseases whose symptoms have been encountered in many tomato fields in Uganda (Defrancq, 1989; Hansen, 1990). Virus-infected plants are typically stunted, crinkled and have small fruits, or no fruits at all. In some cases, infected plants show dieback symptoms before flowering (Hansen, 1990).

Worldwide, of about 146 viruses belonging to 33 genera are reported to infect tomato, a majority 27 viruses belong to genus *Potyvirus*; 17 are Begomoviruses, 15 Nepoviruses, 9 Potexviruses, 8 Tobamoviruses, 6 Luteoviruses, and 6 Tymoviruses, whereas the remaining 67 viruses are distributed among genera *Alfamovirus, Carlavirus, Carmovirus, Closterovirus, Comovirus, Cucumovirus, Cytorhabdovirus, Dianthovirus, Fabavirus, Furovirus, Ilarvirus, Ipomovirus, Luteovirus, Necrovirus, Nucleorhabdovirus, Ourmiavirus, Phytoreovirus, Sequivirus, Tobravirus, Tombusvirus, Tospovirus, Tymovirus, Umbravirus* and *Varicosavirus* (Annex 4)[1].

[1] http://image.fs.uidaho.edu/vide/descr002.htm, 2003

However, not much tomato virus research work has been done in the East African region, partly because of the costs involved in virus identifications. As such, the few available reports from neighbouring countries list only six tomato viruses: *Tomato spotted wilt virus* (TSWV) reported in Tanzania, South Africa, Zambia, Zimbabwe and Madagascar as early as 1969 (CMI, 1969); *Tomato leaf curl virus* (ToLCV) first reported in Sudan in the 1980s (Yassin, 1989); and *Tomato yellow leaf curl virus*-Israel (TYLCV-Is), *Tomato mosaic virus* (ToMV), *Cucumber mosaic virus* (CMV) and *Potato virus Y* (PVY) first reported in Tanzania, Zambia and Malawi in the mid-1990s (Nono-Womdim *et al*., 1996). It is not clear which of these viruses could be infecting tomato in Uganda. Defrancq (1989) observed that probably a complex of tomato viruses occurs in Uganda, and suggested that proper identification should be done as a basis for developing appropriate control technologies. Unfortunately, to the best of our knowledge there was no immediate follow-up on her findings, apart from the general one by Hansen (1990), until the present study was initiated in 1998.

According to Bock (1982) and Green and Kim (1991), it is important that viruses occurring in a specific geographical area are identified and characterized prior to developing sustainable, environment-friendly disease management programmes. Consequently, this study was designed to determine which viruses infect tomato in Uganda.

1.1.2 The Research Problem

Prior to this one, and as reported in 1.2 above, there have been only two general studies on tomato virus diseases in Uganda, i.e. by Defrancq in 1989 and Hansen in 1990. Reports from neighbouring countries indicate the presence of only six viruses, as mentioned in section 1.2 (CMI, 1969; Yassin, 1989; Nono-Womdim *et al*., 1996). It is, however, not clear whether only these viruses, which are found in neighbouring countries or many more of the 146 viruses reported to infect tomato worldwide (http://image.fs.uidaho.edu/vide/descr002.htm) occur in Uganda. Likewise, information

on their incidence in tomato fields in Uganda is lacking. This study therefore, aimed at achieving the objectives presented in sub-section 3 below.

1.1.3 Objectives of the Study

1.1.3.1 Overall Objective (Goal)

The study aims at generating information needed to develop environment-friendly and sustainable tomato virus disease management packages.

1.1.3.2 Specific Objectives

a- To identify and establish incidence of tomato viruses occurring in major tomato growing agro-climatic zones of Uganda;
b- To study the relationship between the tomato virus (es) found to be a major problem in Uganda with similar tomato virus (es) found elsewhere in the world;
c- To establish temporal and spatial spread, as well as the relationship between the tomato virus (es) found to be a major problem in Uganda with their vector (s) in a selected tomato agro-ecosystem.

1.1.4 Hypotheses

a-Several viruses, including those already reported elsewhere in East Africa, namely PVY, PVX, PVMV, CMV, ToMV, TSWV, AMV and TYLCV (CMI, 1969; Yassin, 1989; Chiang *et al*., 1996; Nono-Womdim *et al*., 1996; Czosnek and Laterrot, 1997), infect tomato in Uganda.
b-*Tomato yellow leaf curl virus* (TYLCV-Is) reported to be a big problem in Tanzania (Nono-Womdim *et al*., 1996), could be a problem to tomato growers in some parts of Uganda.

c-TYLCV, suspected to be a problem in Uganda, is not different from tomato yellow leaf curl viruses (*sensu lato)* occurring elsewhere in the world, and these relationships can be established using partial sequences (Brown, 1997).

d-Tomato leaf curl viruses are among the major viruses on tomato and their incidence and spread are influenced by the occurrence and population dynamics of the whitefly vector (*Bemisia tabaci*) (Moriones and Navas-Castillo, 2000), as well as weather conditions in the agro-ecosystem.

e- TYLCV control is possible through whitefly vector management using knowledge of its population dynamics throughout the year, cultural practices and chemical control in an integrated package (Chan and Jeger , 1994; Perring *et al*., 1999).

1.1.5 Description of the Thesis

This thesis has six chapters. Chapter one is the general introduction, which gives the background, research problems, objectives and hypothesis. Chapter two deals with an overview of tomato viruses with specific attention to geminiviruses, their transmission and management. In chapter three, we focus on solving identified problem(s) by generating information on causal organisms of observed viral disease symptoms. Based on the literature review in chapter two, a report on virus-symptom diversity, identification and single or mixed incidence of causal tomato viruses in Uganda, is given. Furthermore, attention is drawn to some new viruses and alternative hosts identified in Uganda. With leaf curl as the most prevalent symptom, and seemingly causing more loss, chapter four goes deeper into characterization of tomato leaf curl and associated mosaic or mottling diseases, which could not be identified using serological techniques. Two viruses, TYLCV-UG and ToLCV-UG, which were identified using molecular techniques, are analyzed and discussed here. Comparison with other geminiviruses whose sequences exist in the European Molecular Biology Laboratory (EMBL), UK and in the National Center for Biotechnology Information (NCBI) GenBank, USA is expounded on, and ToLCV-UG is presented as a new begomovirus. Given that geminivirus-associated leaf curl symptoms are widespread and their known vector *Bemisia tabaci* is common,

attempts are made in chapter five to study the relationship between the two within a selected tomato agro-ecosystem. Chapter six finally presents a summary of the general discussion of key findings and culminates in a series of recommendations, as well as suggestions for future research.

CHAPTER 2

LITERATURE REVIEW

This chapter reviews tomato viruses, their taxonomy, transmission and management with specific emphasis on tomato leaf curl viruses

2 CHAPTER 2

2.1 Literature Review

2.1.1 Virus Taxonomy

In 1970, the International Committee on Taxonomy of Viruses (ICTV) approved sixteen groups of plant viruses. By 1990, Brunt *et al.* (1990) reported 36 plant infecting virus groups to occur worldwide. Matthews (1991) later reported 590 viruses to have been identified and ascribed to 35 families or groups of plant infecting viruses. Following considerable controversy regarding the taxonomy of viruses (Brown, 1997), ICTV later classified viruses into 233 genera (Pringle, 1999) using four criteria: (1) the general nature of the viral genome; (2) the stranded nature of the viral genome; (3) the facility for reserve transcription; and (4) the polarity of the virus genome. Of the 233 genera, 204 were classified into 64 families, leaving 29 genera that were not yet fully characterized (Pringle, 1999). Recently in 2005, Mayo and Brunt reported that ICTV had come up with new approvals for plant virus taxonomy, which include 18 virus families, 82 genera and 17 unassigned genera. Of the 18 families, seven families include viruses that infect tomato, i.e. families *Geminiviridae, Bunyaviridae, Potyviridae, Flexiviridae, Bromoviridae, Closteroviridae,* and *Luteoviridae*. By 2000, ICTV produced the orthography for all known plant viruses. The orthography for tomato viruses belonging to virus families reported above is shown in Table 2.1 (Mayo, 2000).

Furthermore, the geminivirologist community and the family *Geminiviridae* study group under ICTV recognized the existence of the genus *Topocuvirus* (Fauquet *et al*., 2003), in addition to the previously distinguished genera *Mastrevirus*, *Curtovirus* and *Begomovirus*. Member viruses of genus *Mastrevirus* infect monocotyledonous plants like maize (*Zea mays*), and the type species is *Maize streak virus.* On the other hand, member viruses of genus *Curtovirus* infect dicotyledonous plants like beet (*Beta vulgaris*), with

Beet curly top virus, which is the type species. Although members of genus *Begomovirus* infect dicotyledonous plants like tomato (*Lycopersicon esculentum*), they differ from other genera by having twin particles. The species type for this genus is *Bean golden mosaic virus*. Both begomoviruses and tocupoviruses infect tomato, but genus *Tocupovirus* has only one member virus, i.e. *Tomato pseudo curly top virus*, which has a monopartite genome different from that of members of genus *Begomovirus* (Fauquet *et al*., 2003). In the same paper guidelines for naming geminiviruses are outlined. This work (Chapters 3-6) follows 2005 ICTV recommendations (Mayo and Brunt, 2005).

2.1.1.1 Tomato Viruses

About 146 viruses infect tomato worldwide (Green, 1991; http://image.fs.uidaho.edu/vide/descr002.htm). They are grouped into 33 genera, but 15 genera are of the most economic importance, i.e. *Alfalfamovirus, Begomovirus, Carlavirus, Crinivirus, Cucumovirus, Ilarvirus, Luteovirus, Nepovirus, Potexvirus, Potyvirus, Tobamovirus, Tombusvirus, Topocuvirus, Tospovirus,* and *Tymovirus*. As stated before (Section 2.1), these fifteen genera belong to families *Bromoviridae, Bunyaviridae, Closteroviridae, Flexiviridae, Geminiviridae, Luteoviridae* and *Potyviridae* (Pringle, 1999). Family *Bunyaviridae* has only one assigned plant-infecting genus (*Tospovirus*) to which *Tomato spotted wilt virus* (TSWV) belongs. Other genera of this family consist of virus species that infect animals only. Family *Flexiviridae* has been recently approved by ICTV (Mayo and Brunt, 2005). Its major tomato virus is *Potato virus X*, which belongs to genus *Potexvirus*.

Nono-Womdim *et al.* (1994) reported that major tomato viruses in tropical Africa fall into five genera, i.e. *Tobamovirus, Cucumovirus, Tospovirus, Begomovirus*, and *Potyvirus*. No virus was evidenced to be of the genera *Alfamovirus, Potexvirus,* or *Closterovirus,* which have been reported to occur on tomato in Europe (Fauquet and Mayo, 1999: Wisler, 1998; Green, 1991; Brunt *et al*., 1990).

Table 2.1: The orthography of some tomato virus taxon names

Genome	**Before 1995** (Francki *et al*., 1991; Brunt *et al*., 1990; Gibbs *et al*., 1976)		**After 1995 – 2005** (Mayo, 2000; Pringe, 1999; Fauquet & Mayo, 1999; Murphy *et al*., 1995; Mayo and Brunt, 2005)		
	Virus Name	**Group**	**Species**	**Genus**	**Family**
DNA	Tomato yellow leaf curl virus (TYLCV)	Geminivirus	*Tomato yellow leaf curl virus* (TYLCV)	*Begomovirus*	*Geminiviridae*
DNA	Tomato leaf curl virus (ToLCV)	Geminivirus	*Tomato leaf curl virus* (ToLCV)	*Begomovirus*	*Geminiviridae*
(-)ss RNA	Tomato spotted wilt virus (TSWV)	Tospovirus	*Tomato spotted wilt virus* (TSWV)	*Tospovirus*	*Bunyaviridae*
(+)ss RNA	Cucumber mosaic virus (CMV)	Cucumovirus	*Cucumber mosaic virus* (CMV)	*Cucumovirus*	*Bromoviridae*
(+)ss RNA	Tomato aspermy virus (TAV)	Cucumovirus	*Tomato aspermy virus* (TAV)	*Cucumovirus*	*Bromoviridae*
(+)ss RNA	Alfalfa mosaic virus (AMV)	Alfalfa mosaic virus	*Alfalfa mosaic virus* (AMV)	*Alfamovirus*	*Bromoviridae*
(+)ss RNA	Potato virus Y(PVY)	Potyvirus	*Potato virus Y* (PVY)	*Potyvirus*	*Potyviridae*
(+)ss RNA	Tomato mosaic virus (ToMV)	Tobamovirus	*Tomato mosaic virus* (ToMV)	*Tobamovirus*	Unassigned genus
(+)ss RNA	Potato virus X (PVX)	Potexvirus	*Potato virus X* (PVX)	*Potexvirus*	*Flexiviridae*
(+)ss RNA	Pepper veinal mottle virus (PVMV)	Potyvirus	*Pepper veinal mottle virus* (PVMV)	*Potyvirus*	*Potyviridae*
(+)ss RNA	Chili veinal mottle virus (ChiVMV)	Potyvirus	*Chili veinal mottle virus* (ChiVMV)	*Potyvirus*	*Potyviridae*
DNA	-	-	*Tomato infectious chlorosis* (TICV)	*Crinivirus*	*Closteroviridae*

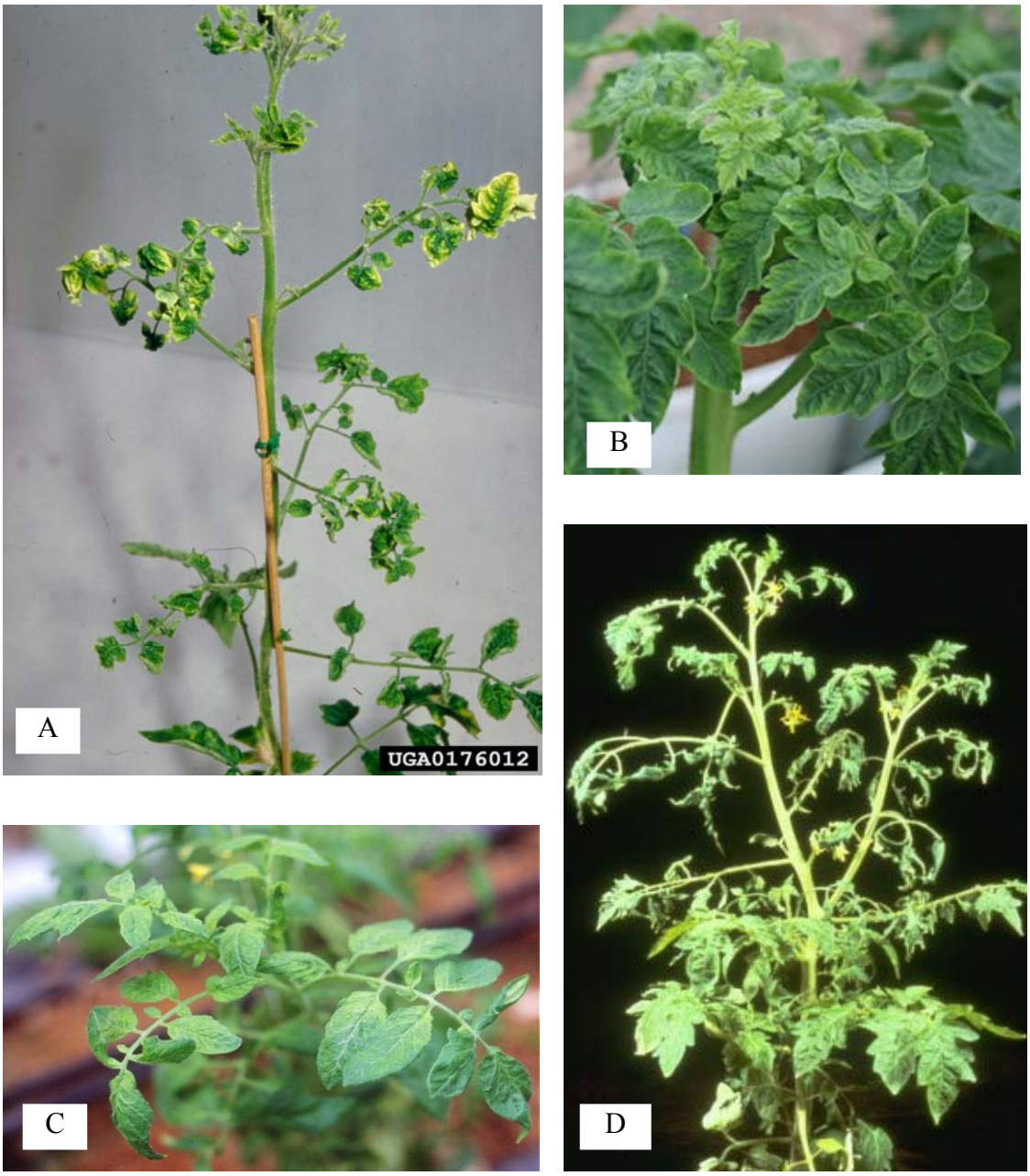


Figure 2.1: Various virus symptoms reported on tomato: A) Tomato vine with severe yellow leaf curl symptoms associated with TYLCV (www.forestry image.org), B) Tomato shoot leaves showing marginal yellowing symptoms associated with TYLCV (www.avrdc.org), C) Tomato shoot with mild mosaic symptoms associated with CMV (www. bspp.org.uk), D) Tomato vine showing crinkling and shoe string like symptoms also associated with CMV (www.inra.fr)

In general, major tomato viruses so far encountered in tropical Africa form two groups based on symptoms (Figure 2.1). The two groups are leaf curl-causing viruses, and mosaic/mottling symptom-causing viruses (Brunt *et al.*, 1990).

2.1.1.1.1 Tomato Leaf Curl Symptom Causing Viruses

Viruses that cause tomato leaf curl, yellowing and chlorosis, and that are transmitted by whiteflies, belong to families *Geminiviridae* and *Closteroviridae*. Viruses of the family *Geminiviridae* cause mostly leaf curl, small round leaflets and marginal yellowing (Czosnek and Laterrot, 1997; Cohen and Nitzany, 1966), whereas those of the *Closteroviridae* induce infectious chlorosis (Wisler *et al*., 1998).

2.1.1.1.1.1 Family *Geminiviridae*

According to ICTV, this family is divided into four genera: *Mastrevirus, Curtovirus*, *Begomovirus* and *Topocuvirus* (Pringle, 1999). The first two genera include viruses infecting maize (*Maize streak virus*) and beet (*Beet curly top virus*), respectively. Members of genera *Begomovirus* and *Topocuvirus* infect tomato. Genus *Begomovirus,* to which *Bean golden mosaic bigeminivirus* belongs as the type species, is the only genus of family *Geminiviridae* that has viruses infecting tomato in both the New and Old Worlds. Genus *Topocuvirus* has only one member, *Tomato pseudo curly top virus*, which is the type species. It has a monopartite genome and also infects dicotyledonous plants.

Geminiviruses have either a monopartite genome or a bipartite genome. According to Brown (1997), monopartite genomes have sizes of 2.7 to 2.8 kb and contain at least six genes. On the other hand, bipartite genomes are 5.2 to 5.4 kb in size, and have two genomic components, named A and B or DNA 1 and DNA 2. Geminiviruses with the bipartite genome were especially reported in the New World (Davies *et al.*, 1989; Rochester *et al.*, 1994).

2.1.1.1.1.1.1 Genus *Begomovirus*

Viruses in this genus were recognized in 1978 by ICTV as forming a distinct class with a specific size, a geminate appearance, and with one or two species of single-stranded DNA. Russo *et al.* (1980) confirmed the presence of geminate particles in tomato tissues. Later, Czosnek *et al.* (1988) isolated and purified TYLCV-Is particles of 20 nm x 30 nm. Navot *et al.* (1991) reported that TYLCV had a monopartite, circular, single-stranded DNA genome of 2.8 kb, which is encapsulated by a coat protein. It has 2,787 nucleotides with six open reading frames (ORFs), two viral-sense and four ORFs on the complementary strand (Novot *et al.*, 1991). Other scientists in Sardinia, and Italy (Kheyr-Pour *et al.*, 1991) confirmed these findings.

Tomato Yellow Leaf Curl Viruses

The major tomato virus in this group of viruses having monopartite single-stranded DNA is *Tomato yellow leaf curl virus* (TYLCV) (*sensu stricto*). Symptoms caused by this virus are chlorotic and leathery leaves, leaf curling, blistering, reduced leaf size, shortened internodes, chlorosis of leaf margins, rounding of leaflets, flower abscission and poor bearing (Cohen and Nitzany, 1966: Yassin, 1982; Makkouk *et al.*, 1983; Thomas, 1984).

According to Padidam *et al.* (1995), there are three distinct TYLCVs based on nucleotide sequence comparisons. It is also considered that viruses of the genus *Begomovirus,* which have nucleotide sequence similarity levels below 90 % are distinct from each other (Padidam *et al*., 1995), although later on ICTV reported that this can only be concluded when complete genome sequences have been compared (Fauquet *et al*., 2003), and not on the basis of the intergenic region (IR) or coat protein gene alone. Similarity comparisons have previously been done on the basis of the intergenic region and partial sequences for other TYLCVs including isolates from Egypt and Israel, which are similar but different from isolates from Spain (GenBank No. L 277081) and Sicily (GenBank No. Z28390) (Noris *et al*. 1993; Antignus and Cohen, 1994), so there is need to characterize and

compare TYLCV isolates from Uganda, if any, with other isolates. It has been stated that there are still many unidentified tomato leaf curl viruses, which are possibly members of genus *Begomovirus* (Jones *et al*., 1991), and this has to be researched and confirmed.

Detection of Tomato Yellow Leaf Curl Viruses (sensu lato)

Serological tests have played a big role in identifying tomato yellow leaf curl viruses. They are widely used, but have limitations because of the need to obtain sufficient purified coat protein for the production of antisera (Czosnek *et al.*, 1988; Credi *et al.*, 1989; Chiemsombat *et al.*, 1991). Initially, polyclonal antibodies were used until Harrison *et al*. (1991) and others begun using monoclonal antibodies. They found that the advantage of using monoclonal antibodies is such that (1) though different viruses share some epitopes, there are those that are specific to one particular virus; and (2) geminiviruses from the same geographic areas tend to share more epitopes than viruses from different regions. This, therefore, served as a basis for use of monoclonal antibodies to study relationships among geminiviruses using monoclonal antibodies raised against *African cassava mosaic virus* (ACMV) (Macintosh *et al.*, 1992).

However, PCR is now more widely adopted because of easy application, sensitivity and specificity for detection and identification of geminiviruses in epidemiological and disease management studies. Navot *et al*. (1992) were able to amplify the genomic DNA molecule of an Israel isolate of *Tomato yellow leaf curl virus* (TYLCV-Is) from total DNA extracts of TYLCV-infected plants. Rojas *et al.* (1993) took advantage of geminiviruses replicating via a double-stranded, circular DNA form to characterise bi-partite geminiviruses from the Americas. Through the PCR process, a specific DNA fragment that lies between two primer-annealing points is amplified. Degenerate (general) primers are used for general amplification of part of the viral genome sequence. Oligonucleotide (specific) primers, which anneal to either C1 or V1, are used for specific amplification of desired fragments of TYLCV DNA sequence (Nakhla *et al.*, 1994).

Therefore, both degenerate and specific primers could be used to identify and characterise TYLCV occurring in Uganda.

A more recent and improved PCR now exists. It employs more than one primer pair, mostly specific ones, to target specific parts of the replication gene and intergenic region. This method is called multiplex PCR (Potter *et al*., 2003; Gorsane *et al*., 2005), and it is reported to be faster and even cheaper than the PCR technique described above.

Distribution of Tomato Yellow Leaf Curl Viruses

TYLCV is quite general in the tropics. In Africa, it has been reported from South Africa, Senegal, Tanzania, Malawi, Zambia, Zimbabwe, Nigeria, Ivory Coast, Egypt and Sudan (Yassin *et al.*, 1982; AVRDC, 1987; Czosneck *et al.*1990; Nakhla *et al.*, 1993; AVRDC, 1993; Nono-Womdim *et al*, 1994; Chiang *et al*., 1996). It is also widespread in the rest of the Old World and in the New World, e.g. in South East Asia and East Asia, the Americas and the Mediterranean (Green and Kallo, 1994; Chiang *et al.*, 1996; Polston and Anderson, 1997; Czosnek and Laterrot, 1997). Therefore, it is likely that the virus also occurs in Uganda, since it affects tomato in neighbouring countries, which have similar climatic conditions. This suggests the need to investigate the presence and status of TYLCV in Uganda.

Transmission of Tomato Yellow Leaf Curl Viruses

TYLCV is transmitted by a whitefly (*Bemisia tabaci* Gennadius) of the Family *Aleyrodidae* (Cohen and Nitzany, 1966; Nakhla *et al.*, 1978; Gerling and Mayer, 1995). *Bemisia tabaci* occurs in biotypes A and B. Biotype B is more common than A and is regarded by some as a separate species designated *B*. *argentifolii* (Bellows *et al.*, 1994). Others continue to regard it as a biotype of *B. tabaci*, even though there are many more biotypes, which include biotype Q (Demichelis *et al*., 2000).

In some circumstances, the incidence and rate of spread of TYLCV are directly proportional to the whitefly population present in the environment (Mansour *et al.*, 1992; Mehta *et al.*, 1994). Both adults and larvae can acquire the virus by feeding on infected plants with a minimum access and acquisition period (AAP) of 15 minutes. The virus has a latent period of 21-24 hours, and persists for 10 to 20 days in viruliferous *B. tabaci* adults (Cohen *et al.*, 1966; Zeidan and Czosnek, 1994). For the whitefly to transmit the virus persistently, it must have adequate inoculation access periods (IAP) following acquisition access periods (AAP) (Cohen *et al.*, 1964).

According to Zeidan and Czosnek (1994), TYLCV multiplies inside *B. tabaci*. Cohen and Antignus (1994) demonstrated that TYLCV could be found as double-stranded DNAs in viruliferous whiteflies, which implies replication of viral DNA in the vector. Once the whitefly has acquired the virus, it can continue transmission through out its life (Brown, 1997). These observations are opposed to earlier findings (Cohen and Nitzany 1966), which stated that TYLCV triggers an antiviral mechanism in *B. tabaci,* hence preventing multiplication of the virus in the vector and necessitating the need for repeated acquisition (Cohen and Harpaz, 1964). In another study, Marco *et al.* (1975) found anti-TMV factors that influenced periodic acquisition of the virus, and reduce whitefly ability to acquire and transmit viruses. According to Briddon *et al.* (1990) and Hiebert *et al.* (1995), factors involved in virus transmission include presence of viral coat protein and genes on the complementary DNA strand with open reading frames ACI, AC2, AC3, and AC4.

The presence of viral DNA in the vector is proof of vector transmission of that particular virus (Navot *et al.* (1992). It can be determined by using modern techniques like Polymerase Chain Reaction (PCR). Navot *et al.* (1992) used specific primers to achieve high level DNA amplification in PCR experiments. However, the best approach for use in developing countries with limited research facilities would be the use of DNA hybridisation to check for viral DNA in plants and whiteflies (Czosnek *et al.*, 1988;

Navot *et al.*, 1991). Even though serological techniques are the easiest, they have limited sensitivity (Credi *et al.*, 1989; Chiemsombat *et al.*, 1991).

While assessing other methods of TYLCV transmission, Makkouk (1979) established that direct contact between plants, natural root grafting through adjacent roots, seed infection, and soil contamination are not effective in transmitting TYLCV, and that the only efficient method of transmission is by *B. tabaci* or *B. argentifolii*. The different views on TYLCV transmission indicate the need for further investigation of the subject.

Host Range of Tomato Yellow Leaf Curl Viruses

Tomato yellow leaf curl virus (*sensu lato*) has a very wide host range. Thus, Nono-Womdim *et al.* (1996) detected TYLCV in a number of weed species in Tanzania, which are alternative TYLCV hosts. Concurrently, Legg (1996) found that *B. tabaci* selectively colonises cassava, sweet potato and cotton in Uganda. He established cultures of *B. tabaci* on cassava, sweet potato and cotton in the laboratory. Earlier, Butler *et al*. (1986) reported whitefly cultivars oviposition preference for cotton. In addition to tomato, the following plants have been reported as hosts of tomato yellow leaf curl viruses:

Family Solanaceae*: Capsicum annuum, C. frutescens, Datura stramonium, D. bernhardii, Lycopersicon peruvianum, L. hirsutum, L. pimpinellifolium, Nicotiana sylvestris, N. benthamiana, N. glutinosa,* and *Nicotiana tabacum vars Samsun* and *Havana 423, and Solanum nigrum*
Family Malvaceae*: Malva arvensis, Malva nicaensis, M. parviflora, Corchorus tinctorius, Hibiscus syriacus,* and *Gossypium hirsutum*
Family Fabaceae*: Arachis hypogaea, Lens esculenta, and Phaseolus vulgaris*
Family Pedaliaceae: *Sesamum indicum.*
Family Asteraceae*: Sonchus oleraceus*
Family Euphorbiaceae*: Euphorbia heterophylla*
Family Acanthaceae*: Achyranthes aspera*

Others*: Chaerogphyllum* spp*., Cynanchum acutum, Hyoscyamus desertorum, Nicandra physaloides,* and *Vitis vinifera* (Cohen and Antignus, 1994; Nakhla *et al.*, 1994; Mansour, 1992; Ioannu, 1987; Nakhla *et al.*, 1978; Nitzany, 1975; Cohen and Nitzany, 1966; Nono-Womdim *et al.*, 1996).

Cohen and Antignus (1994) used viruliferous *B. tabaci* for inoculation of plants belonging to diverse families, such as Asclepiadaceae, Asteraceae, Fabaceae, Malvaceae, Solanaceae and Apiaceae in the greenhouse, and virus-free whiteflies for recovery tests, to determine TYLCV host range. The extensive host range has consequences for virus disease epidemiology and is worth considering while developing a system-wide management strategy for these viruses. Furthermore, it is doubtable whether crop rotation is a feasible tomato yellow leaf curl virus diseases control measure.

Tomato Yellow Leaf Curl Viruses (sensu lato) Epidemiology

Epidemiological studies by Moustafa (1991), in the semi-tropical climatic zone of Egypt, indicated that at the beginning of Spring and early Summer (February - April), when tomato plants have just established, TYLCV incidence is very low. The latter becomes high towards the end of Summer (September – mid-October), and then coincides with peak whitefly population density (Riley *et al.*, 1995). This is followed by high TYLCV incidence and severe damage in the fall (Autumn) when production losses rise to 80% and almost all plants are infected. Similarly, Cohen and Antignus (1994) observed that in the Jordan Valley, the spread of TYLCV was significantly correlated with *B. tabaci* population size. As in Egypt, peak whitefly population occurred between the first week of September and Mid-October. In Tanzania, TYLCV symptoms and whitefly vector presence are reported to be most common during November to February (Nono-Womdim *et al*., 1996).

Another factor contributing to high incidence of TYLCV is proximity to old host crop fields. Mazyad *et al.* (1994) found that adjacent old fields of vegetables and other field

crops present at tomato planting play a big role in harbouring whiteflies, which eventually infest tomatoes. Similar observations were made in Egypt, Cyprus, Lebanon, Jordan, Saudi Arabia and Israel (loannou *et al.*, 1991; Mazyad *et al.*, 1986; Makkouk *et al.*, 1979; Nitzany, 1975). Although some whitefly vector-host studies have been conducted in relation to Cassava mosaic geminiviruses (CMGs) in Uganda (Legg, 1996), there is a need to establish the relationship between whitefly vector and transmitted TYLCV in order to develop sound TYLCV management options.

Management of Tomato Yellow Curl Virus

The most effective way of managing *Tomato yellow leaf curl virus* (*sensu stricto*) is by use of an integrated management package, which combines cultural practices, insecticide application, UV-absorbing plastic films, insect vector proof nets, and variation of weather conditions like light intensity, photoperiod and temperature (loannou *et al.*, 1985; Mazyad *et al.*, 1986; Cohen and Antignus, 1994; Perring *et al*., 1999; Palumbo *et al*., 2001; Hilje *et al*., 2001; Mutwiwa *et al*., 2005; Zilahi-Balogh *et al*., 2006; Kumar *et al*., 2006). Mazyad *et al.* (1986), and Chan and Jeger (1994) controlled TYLCV by eliminating or reducing sources of initial inoculums through uprooting diseased plants. Chan and Jeger (1994) reported that uprooting was most effective where plants were sparsely distributed and with minimal contact. This may also relate to young plants whose canopies are still small. Ioannou *et al.* (1987) recommended the use of healthy transplants, and later in 1991 reported successful control of TYLCV by timely planting. Thus, the whitefly population is low during the rainy season and high in the dry season. Careful tomato seedling protection against whiteflies in the dry season, and transplanting at the beginning of the rainy season helped to avoid TYLCV infection. Sharaf *et al.* (1986); Prabhaker *et al.* (1988); Mason *et al*. (2000); and Palumbo *et al*. (2001) managed vector populations by using chemical pest control options, such as Imidacloprid (a nicotinoid), buprofezin (a chitin synthesis inhibitor), and pyriproxyfen (a juvenile hormone analog). However, Palumbo *et al*. (2001) recommend use of cultural and biological options for controlling *B.tabaci* because of pest resistance to insecticides.

Cohen and Antignus (1994), Mazyad *et al.* (1994), as well as Greer and Dole (2003) used cultural practices, such as yellow traps and mulches, intercropping tomato with other crop species, and physical barriers to reduce whitefly movement. Kasrawi *et al.* (1988), Lapidot and Friedmann (2002), Rubio *et al.* (2003), Yang *et al*. (2004), de Castro *et al*. (2005), and Fuentes *et al*. (2006) aimed at controlling TYLCV by breeding or engineering for resistance, while Berlinger and Dahan (1985), and Kisha (1984) researched into host plant resistance to the whitefly vector. All these approaches depend on accurate virus identification to avoid targeting a wrong pathogen (Hamilton *et al*., 1981; Bock, 1982).

2.1.1.1.1.2 Family *Closteroviridae*

This family includes two genera, *Closterovirus* and *Crinivirus*. A virus, which causes infectious tomato chlorosis, was attributed to the genus *Closterovirus* (Wisler, 1998). However, the International Committee for the Taxonomy of Viruses (ICTV) later classified it as a member of genus *Crinivirus* (Fauquet and Mayo, 1999). As such, genus *Closterovirus* has no yet known tomato virus.

2.1.1.1.1.2.1 Genus *Crinivirus*

Whiteflies transmit criniviruses. These viruses are RNA flexuous filamentous rods, 600 – 2000 nm long. Closteroviruses cause general leaf yellowing, vein chlorosis and phloem necrosis in susceptible hosts (Duffus, 1995). They are reported to be associated with intracellular inclusions and vesicles. Their coat protein has a molecular weight ranging from 23 kD – 27 kD, whereas their nucleic acid (RNA) has a molecular weight of 2,000 kD - 4,500 kD (Duffus, 1995). A member of this genus that infects tomato is the *Tomato infectious chlorosis virus* (TICV).

Tomato Infectious Chlorosis Virus (TICV)

Tomato infectious chlorosis virus (TICV) is a recently identified tomato virus from California (Duffus *et al*., 1994). It causes interveinal yellowing, necrosis and severe yield losses. These symptoms are easily confused with those of TYLCV. TICV is transmitted from tomato to tomato, as well as from wild plants in the tomato ecosystem by the whitefly species *Trialeurodes vaporariorum*, and has flexuous filamentous particles of variable length, and a bipartite RNA (Wisler *et al.*, 1998).

Tomato Infectious Chlorosis Virus Host Range

The host range of TICV includes *Geranium dissectum* (L.), *Lycopersicon esculentum* (Mill.), *Petunia* spp., and *Ranunculus asiaticus* (L). Other hosts are (Duffus, 1994):
Family Chenopodiaceae: *Chenopodium murale* (L.), *C. capitatum* (L.).
Family Asteraceae: *Lactuca sativa* (L.), *Senecio vulgaris* (L.), *Sonchus oleraceus* (L.), *Zinnia elegans* (J.).
Family Solanaceae: *Nicotiana benthamiana* (D.), *N. clevelandii* (G.), *N. glauca* (G.), *Petunia hybrida* (V.), *Physalis alkekengi* (L.), *P. floridana* (R.), *P. ixocarpa* (B.), *P. wrightii* (G.), *Solanum tuberosum* (L.)
The presence of TICV in weeds was reported to influence development of appropriate management packages (Wisler *et al.*, 1998).

Tomato Infectious Chlorosis Virus Management

Tentative control measures for TICV recently identified in the USA and Italy, are (1) creating farmer awareness of disease symptoms; (2) uprooting of infected plants; (3) rotation with non-susceptible crops; and (4) chemical control of whiteflies (Wisler *et al.,* 1997). In the process, Wisler *et al.* (1997) further indicated that re-enforcement of quarantine regulations was necessary to avoid introduction of new viruses and spreading them from country to country.

2.1.1.1.2 Mosaic and Mottling Symptom-causing Viruses

Viruses belonging to the following six families: *Tombusviridae, Luteoviridae, Potyviridae, Flexiviridae, Bromoviridae* and *Bunyaviridae* are known to cause mottling and mosaic symptoms on tomato. Symptoms caused by these viruses vary from white mosaic to yellow mosaic and sometimes express themselves as mild green mottling (Brunt *et al.*, 1990). A detailed account of each of these families follows. However, genus *Tobamovirus* is not yet attributed to any virus family, and therefore stands on its own (Mayo, 2000).

2.1.1.1.2.1 Genus *Tobamovirus*

These viruses have elongated particles of 300 x 18 nm and contain RNA molecules. Viral particles are found in trichomes and epidermal cells of infected plants and occur in hexagonal, crystalline arrays (Green and Kim, 1991). *Tobacco mosaic virus* (TMV) is the type species of this genus. Another species of this genus is *Tomato mosaic virus* (ToMV), which is distinguished from TMV by its ability to produce local necrotic lesions in *Nicotiana tabacum* var. White Burley and *N. sylvestris* (Green and Kim, 1991). ToMV strains include those, which cause corky ring, crusty fruit, yellow streak and aucuba symptoms (Kang *et al*., 1981; Jones *et al.*, 1991). Consequently, it is not easy to correctly identify ToMV by basing on symptoms because it causes a variety of them. However, known common ToMV symptoms include mosaic, systemic chlorosis, local necrotic lesions, leaf abscission, as well as systemic leaf and stem necrosis, which ultimately cause death (Brunt *et al.*, 1990; Green and Kim, 1991; Jones *et al.,* 1991).

The virus is transmitted by human activities, through seed, and from leaf and root debris (Green and Kim, 1991). It is also readily sap-transmissible and cosmopolitan (Brunt *et al*., 1990). ToMV has been found as an aerosol in fog in USA (Castello *et al.*, 1995) and in nutrient solution used for crop cultivation in Apulia, Italy (Pares *et al.*, 1992; Gallitelli *et al*., 1982), and in Spain (Cordero, 1983). It has also been reported in Tanzania, Malawi and Zambia (AVRDC, 1987 and 1993; Nono-Womdim, 1994). Consequently,

ToMV is likely to occur in Uganda, where reports of its occurrence are actually based on symptomatology (Defrancq, 1989; and Hansen, 1990) and therefore not very reliable.

2.1.1.1.2.2 **Family *Flexiviridae***

This family has eight genera, which include *Potexvirus*, *Carlavirus*, *Capillovirus*, *Trichovirus*, *Foveavirus*, *Allexivirus*, *Vitivirus* and *Mandarivirus* (Mayo and Brunt, 2005; Adams *et al*., 2004). Its members have flexuous virions. Genera *Carlavirus* and *Potexvirus* are known to infect tomato. The latter is a more important tomato virus genus than the first, even though it is less important than other genera reported in this review.

2.1.1.1.2.2.1 Genus *Potexvirus*

This genus includes tomato-infecting virus species, *Potato virus X* (PVX). PVX is considered to be of less economic importance to tomato production in tropical Africa than other tomato viruses. Nono-Womdim *et al.* (1996), while reporting about other tomato viruses, did not report PVX occurrence in Tanzania, and until this study it was not known whether PVX infects tomato in Uganda.

2.1.1.1.2.3 Family *Potyviridae*

There are six member genera of this family, i.e. *Bymovirus, Ipomovirus*, *Macluravirus, Potyvirus, Rymovirus, Tritimovirus* and (Mayo and Brunt, 2005; Pringle, 1999). Of these, only the genus *Potyvirus* is known to have members that infect tomatoes.

2.1.1.1.2.3.1 Genus *Potyvirus*

This is the largest and economically most important group of plant viruses (Jones *et al.*, 1991). In the VIDE database index of plant viruses (2006), for every 10 virus species listed at least one is a potyvirus. Potyviruses induce typical cylindrical, pinwheel-shaped inclusions in cells of infected plants (Green and Kim, 1991). Some major viruses in this family that infect tomato include *Potato virus Y* (PVY), and *Pepper veinal mottle virus*

(PVMV) (Zitter, 1974; Nono-Womdim, 1994). *Potato virus Y*, a member of the genus *Potyvirus* that infects tomato, has long flexuous rod-shaped particles (730 x 11 nm) containing single-stranded RNA. *Potato virus Y* occurs in several pathotypes (Jones *et al.,* 1991), which are transmitted by aphids in a non-persistent manner.

Typical symptoms of PVY in tomato include mosaic, vein chlorosis, mild mottling, dark brown necrosis on leaflets, severe necrosis, leaf crinkling, and drooping (Jones *et al*., 1991). The virus occurs worldwide, but has a narrow host range in the tropics. Tanzania, Malawi and Zambia are among the few countries where PVY occurs in tomato and pepper (AVRDC, 1985, and 1993; Nono-Womdim *et al.*, 1994). It has also been evidenced in Apulia, Italy in tomato (Gallitelli, 1982). There are, however, no reports of PVMV in tomato in East Africa, although Nono-Womdim *et al.* (1994) reported it in sweet pepper (*Capsicum annum*) in the SADC region.

2.1.1.1.2.4 Family *Bromoviridae*

Family *Bromoviridae* has five genera that infect plants, i.e. *Alfamovirus, Ilarvirus, Bromovirus*, *Cucumovirus* and *Oleavirus* (Pringle, 1999). Two of these genera include viruses known to infect tomato, i.e. genera *Alfamovirus* and *Cucumovirus* (Brunt *et al*., 1990; Jones *et al*., 1991).

2.1.1.1.2.4.1 Genus *Cucumovirus*

Cucumoviruses have three functional pieces of ssRNA, i.e. RNA 1, 2 and 3, which determine virus virulence (Jones *et al.*, 1991). These virus particles are isometric and measure on average 28 nm in diameter (Jones *et al.*, 1991). In addition to the three RNAs, cucumoviruses have a fourth RNA (RNA 4), which acts as a subgenomic coat protein messenger, and also a fifth RNA (CARNA 5) or satellite RNA.

There are two major tomato viruses in this genus: *Cucumber mosaic virus* (CMV) and *Tomato aspermy virus* (TAV). According to Green and Kim (1991) and Jones *et al*.

(1991), of the two species CMV is the most common in the tropics. It causes mottling, mosaic, yellow discolouration, vein-clearing, stunting, and leaf deformation, with extreme filiformity or shoestring leaf appearance. In severe infections, plants produce no fruits or only very few fruits, which are of small size (Green and Kim, 1991; Jones *et al.,* 1991). These cucumoviruses are transmitted in a non-persistent manner by aphids (Green and Kim, 1991). CMV is present in the tropics. In Africa it has been evidenced in Tanzania, Malawi and Zambia (AVRDC, 1987, 1993; Nono-Womdim *et al.,* 1994).

2.1.1.1.2.4.2 Genus *Alfamovirus*

Members of this group, according to Green and Kim (1991) and Jones *et al.* (1991), contain four ssRNAs, with every each RNA consisting of five different components. One of the RNA components is isometric and 18 nm in diameter. The other four components are bacilliform with lengths of 58, 49, 38 and 29 nm, respectively and a width of 18 nm. Vacuole inclusions are found in cell cytoplasm (Green and Kim, 1991; Jones *et al*., 1991). One of the member species is *Alfalfa mosaic virus* (AMV), which infects tomato. AMV causes tomato leaf mottling, interveinal yellowing, yellow whitish mosaic, veinal necrosis, chlorotic line patterns, chlorotic rings and black spots. Fruits of AMV-infected plants are small (Jones *et al.*, 1991). AMV is transmitted by 14 aphid species in a non-persistent manner and through infected seed (Brunt *et al.*, 1990; Green and Kim, 1991; Jones *et al.*, 1991). It occurs worldwide, but before this study no report had been made of AMV in tomato in Uganda.

2.1.1.1.2.5 Family *Bunyaviridae*

The family is divided into five genera, i.e. *Bunyavirus, Hantavirus, Nairovirus, Phlebovirus* and *Tospovirus* (Pringle, 1999). Of these, only genus *Tospovirus* has viruses that infect plants and especially tomato. Other genera have viruses that infect animals.

2.1.1.1.2.5.1 Genus *Tospovirus*

This genus contains viruses, which are very unstable, especially at pH values below 5.5. Viruses of this genus have a characteristic membranous lipoprotein envelope and form cytoplasmatic inclusions in plant cells (Green and Kim 1991). The isometric particles are 70-90 nm in diameter and contain RNA as well as protein particles (Jones *et al.*, 1991). Protein 1 is a nucleoprotein, while proteins 2, 3, and 4 are membrane-associated glycoproteins (Verkleij and Peters, 1983; Gonslaves and Providenti, 1989; Jones *et al.*, 1991; Ullman *et al.*1996). *Tomato spotted wilt virus* (TSWV) is the only known member of this genus that infects tomato. Mild mutant strains of TSWV exist and have been inoculated into tomato for cross-protection against more severe strains (Gonslaves and Providenti, 1989). TSWV is known to cause chlorosis and yellow rings on tomato leaves and fruits.

TSWV is transmitted persistently by thrips. Seed transmission also occurs (Jones *et al.*, 1991; Ullman *et al.*, 1996). TSWV occurs in many countries including Italy (Bellardi and Bertaccini, 1992; Vovlas *et al.*, 1993), Tanzania, South Africa, Zambia, Zimbabwe, and Madagascar where the virus was first identified (CMI, 1969). However, TSWV has not yet been identified in Uganda.

Based on the above reviewed virus research results, there is no hard evidence of identification of viruses, which occur on tomatoes in Uganda. Consequently, this study was undertaken.

2.1.2 Methods Used to Identify Tomato Viruses

Under field situations, the most obvious viral symptoms are mosaic, mottling, necrosis and leaf distortions, but for identification of symptom causing viruses, these features are not very reliable on their own because they are influenced by a number of other factors such as sucking insect pest infestation, and plant-water relations (Green, 1991).

Consequently, several laboratory methods have been developed to identify viruses (Matthews, 1991). These include the use of test plants, serology, and various molecular tests.

2.1.2.1 Test Plants

Test plants are diagnostic tools mostly used to detect sap-transmitted viruses. For example, the World Vegetable Center (AVRDC) in Taiwan uses this kind of technique (Green, 1991). Some examples of test plants include: *Capsicum annuum, C. frutescens, Chenopodium quinoa, C. amaranticolor, Cucumis sativus, Gomphrena globosa, Lycopersicon esculentum, Nicotiana tabacum, N. rustica, Petunia hybrida, Phaseolus vulgaris,* and *Pisum sativum* (Brunt *et al.*, 1990). These test plants are used especially to confirm virus infection, but may not identify the actual virus causing the problem. Therefore, the technique is not very efficient for taxonomy. Moreover, it is time consuming. As such, serological tests are preferred (Bock 1982).

2.1.2.2 Serological Tests

The micro-precipitin serological test was one of the first serological methods used for laboratory identification of viruses (Duncan and Torrance, 1992). It is an expensive test in terms of the need for large quantities of antisera. It is less sensitive than enzyme-linked immunosorbent assay (ELISA), though easier to use. ELISA is a serological test that uses antiserum prepared against a particular virus. The antiserum contains antibodies generated in blood serum of rabbits inoculated with that particular virus' antigen, and can be made in a local and simple laboratory. This antiserum and alkaline buffers are used on microtiter plastic plates to test plant sap for that specific virus (Clark and Adams, 1977).

Another serological test is the Ouchterlony Agar Gel Double Diffusion test (Matthews, 1991). It is a simple test, useful for identifying viruses. It uses crude antisera, which contain immunoglobulin M (IgM) and immunoglobulin G (IgG). Though it is not often available on the market, commercial companies can avail it on special order (Bragard,

2006 personal communication). In addition to the above techniques, immunoelectron microscopy can be used to identify individual viruses occurring in a single plant sap extract at the same time, and also to study virus particle size and shape (Duncan and Torrance, 1992). Furthermore, there is the Double-Antigen-Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) used for immediate serological identification of viruses in a sample, based on viral protein differences (Clark and Adams, 1977). DAS-ELISA is widely used. The reagents and chemicals required are readily available, and it gives adequate identification of viruses. Nono-Womdim and Atibalentja (1993) used DAS-ELISA to identify PVMV in sweet pepper (*Capsicum annuum*) in Cameroon. Like all other ELISAs, it is fairly cheap, especially if antisera can be produced locally and do not have to be bought from commercial companies.

Triple-Antibody-Sandwich (TAS-ELISA) is another form of ELISA. It uses monoclonal antibodies to detect viruses such as tomato geminiviruses (Credi *et al.*, 1989; Roberts *et al.*, 1984; Thomas *et al*., 1986). TAS is efficient and easy to conduct in conditions of limited time and space. Macintosh *et al.* (1992) used TAS to study tomato geminiviruses in Europe. It is more specific than DAS, because of the monoclonal antibodies used. Like DAS, it is fairly cheap (Matthews, 1991), though not as accurate as molecular techniques.

2.1.2.3 Molecular Tests

Recently, molecular techniques have been developed to identify viruses. Polymerase Chain Reaction (PCR) is one of these techniques (Duncan and Torrance, 1992). It is based on differences between viral nucleic acid, and is very efficient, as well as accurate depending on the type of primers used (specific or general) (Lewin, 1997). However, it is an expensive technique using high cost equipment and reagents (Maniatis *et al.*, 1982). Similarly, the nucleic acid hybridisation test is another convenient molecular technique for identifying viruses that cannot be identified by using serology (Czosnek *et al.*, 1988). It is efficient, but uses expensive reagents and chemicals. It requires special expensive laboratory facilities, especially if it uses radioactive probes (P^{32}) and not non-radioactive

(biotin) ones. Furthermore, DNA hybridisation is very useful in that it can be used to test samples sent from normally poorly equipped laboratories in developing countries to advanced laboratories.

One way of quickly differentiating geminiviruses is by sequence pairwise comparison and phylogenies (Padidam *et al*., 1995; Brown, 1997; Fauquet *et al*., 2003). However, there are different opinions. Some scientists feel that sequence comparisons could be based on the intergenic region and coat protein gene (Brown, 1997) in the absence of a complete DNA sequence, while others feel that full sequence comparisons (Fauquet *et al*., 2003) are necessary. In the absence of adequate resources, the former approach to geminivirus identification would be most appropriate for developing countries, whereas complete sequence comparisons would be encouraged, where possible.

Based on results obtained by using the above techniques, and under the coordination of the International Committee for the Taxonomy of Viruses (ICTV), tomato viruses have been characterized and grouped in orders, families, genera and species (Padidam *et al.*, 1997; Fauquet and Mayo, 1999; Pringle, 1999; Mayo and Brunt, 2005).

2.1.3 Virus Epidemiology and Vector Relationships

2.1.3.1 Virus Transmission

Viruses can be transmitted by mechanical means, and by pollen, seeds, fungi, nematodes, mites, as well as insects (Green and Kim, 1991; Green, 1991; Jones *et al.*, 1991), although there is considerable specificity and any one virus is often transmitted by only some of these routes. Mechanical transmission is mostly restricted to non-persistent and semi-persistent aphid-transmitted, leafhopper-transmitted and some whitefly-transmitted viruses (Brunt *et al*., 1990; Green 1991; Jones *et al.*, 1991). Known insect vectors of tomato viruses are beetles, aphids, leafhoppers, thrips and whiteflies (Lyons *et al*, 1985; Duffus, 1987; Black *et al.*, 1991; Green and Kim, 1991 and Green, 1991; Jones *et al.*,

1991; Bautista *et al.*, 1995; Van de Watering *et al.*, 1996). Major vectors of tomato viruses in East Africa are whiteflies, aphids and thrips (Nono-Womdim *et al.*, 1996). Aphids and whiteflies are the major vectors reported in Uganda (Baliddawa, 1990). Of the two vectors, only whiteflies transmit tomato viruses in a persistent manner (Green, 1991), and as such they transmit geminiviruses efficiently from one host to another, tomato included.

It is known that vectors spread viruses from one infected host to another especially due to their transient feeding behaviour. Movement of vectors depends on host range and presence, as well as vector host preference and life history. Doncaster (1943) observed that vectors actively fly from one plant to another in still air, and are less active over long distances when blown by wind, but could still be active without wind, provided wings continue to beat (Thresh, personal communication). Changes in cropping systems and weeding patterns affect vector populations and virus transmission, as has been the case for *Tomato spotted wilt virus* (TSWV) in Hawaii (Gonslaves and Providenti, 1989). According to Raccah (1986), presence of inoculum is an essential factor in virus transmission. Therefore, field environmental factors and insect pest population dynamics should be studied if one is to understand the relationship between transmitted virus and vector.

Raccah (1986) monitored spread of viral infection in space and time by measuring the distance between old and new infections in relation to time interval between detection of first symptom and subsequent symptoms. At each stage of disease spread, the number of plants infected was recorded to develop temporal and spatial patterns of disease-spread curves. Disease spread in space was calculated using an equation developed by Allen (Plumb and Thresh, 1983), in which the probability of a new infection to occur at a distance x from the source was deduced by:

$$Px = 1\text{-}\exp(-x/x^{1}) \qquad (1),$$

whereby x is distance from source of infection; P is the probability, and exp is the exponential factor. Earlier Vanderplank (1963), determined virus spread with time by:

$$dN/dt = KN (Nmax – N) \qquad (2),$$

whereby N is number of plants infected; t is the time; dN is the difference between number of plants infected at a particular time and another; dt is the difference in time; K is a constant factor; and Nmax is the maximum number of plants infected.

Plumb and Thresh (1983) and Raccah (1986) also studied virus spread in time and space in relation to prevailing weather conditions. They found that there was direct influence of weather conditions on the rate at which viruses spread.

According to http://www.apsnet.org (2003), if disease progress is a monocyclic epidemic, and is linear, the slope of the disease progress curve is constant. Furthermore, disease progress in a monocyclic epidemic is proportional to the amount of initial inoculum. We can calculate the slope of the disease progress curve, and describe a monocyclic epidemic with linear disease progress curves using the differential equation:

$$dx/dt = QR \qquad (3),$$

whereby dx is an infinitesimally small increment in disease severity; dt is an infinitesimally small time step; Q is the amount of initial inoculum; and R is a proportionality constant that represents the rate of disease progress per unit of inoculum. Since Q and R are both constant during the course of an epidemic, the slope, dx/dt, is constant, and disease progress is linear. R has a value that represents the "average" for the whole epidemic, a value that depends on many factors such as aggressiveness of pathogen, host susceptibility, environmental conditions, etc.

The units of R are proportional to initial inoculum unit per unit time, if we integrate the above differential equation by:

$$x = QRt \qquad (4),$$

Furthermore, according to Thresh (1998), a virus disease tends to spread until a saturation level when there are no more plants to infect (100% infected). He went on to explain that disease spread tends to be arithmetic and not logarithmic for several reasons: (1) crop fields are finite; (2) there are spatial constraints; (3) host plants vary; (4) mature plants develop resistance; and (5) there are experimental effects imposed by applied treatments. Thresh (1998) indicated that spread is most effective from the local inoculum, which already exists on a few plants, outwards to the nearest hosts. He reported that experimental treatments could delay the build-up of initial inoculum and/or slow down disease spread. Thus, a susceptible variety would be infected at a higher rate than a resistant variety. Furthermore, disease occurrence and virus inoculum concentration are not directly proportional. The inoculum can occur as scattered foci, in which case virus inoculum is high or else occur as single foci, in which case virus inoculum concentration is considered to be low. Scattered foci are more damaging than single foci, because they are in contact with more healthy neighbours. Thresh (1998) further indicated that from the initial foci, spread can be either monocyclic or polycyclic. Monocyclic spread involves only one stage of disease spread, in that the disease develops on a plant, which eventually dies without the disease spreading out to other plants. A monocyclic situation arises when the vector is able to transmit a virus from weed foci to the crop, and not from plant foci to neighbouring plants within the crop. This then results into a localized high rate of infection at the early stages, which levels off and drops with time. Polycyclic disease spread involves successive cycles of disease spread, in that a disease that started with a few plants spreads out to other plants until the whole crop is infected. Polycyclic disease spread results into a sigmoid curve. Its upper limit is determined by host maturity, weather or lack of susceptible hosts. The latter is a more usual disease spread situation than the former. Thresh (1998) reported virus spread to be either from the crop itself

(crop foci), or from an adjacent or distant field. Spread within the crop takes place whether virus transmission is persistent or non-persistent, while spread from distant fields is possible especially for persistent viruses. Thresh (1998) went further to report that if infection decreases from peripheral field rows to the centre, then the source of infection is outside the field. He further indicated that the smaller the field plot, the more the external influence of peripheral rows. Thus, an elongated field (rectangle) will be more affected than a square field having the same surface area. By considering the different biotic agents of disease spread, Thresh (1998) indicated that the more mobile the vector, the longer the risk distance along which it can transmit the disease. The risk distance is the path along which the vector that has acquired inoculum is able to move while still with potential to transmit the virus to other plants. The latter author also explained that planting in line and parallel to the prevailing wind direction could be expected to lead to less infection, because most vectors are blown away by wind. When planting is done in lines across the prevailing wind direction, vector movement is reduced.

Sigiura and Bandaranayake (1975) working on viruses of chilli pepper found that two aphid genera are associated with virus transmission, i.e. genus *Aphis* and genus *Myzus*. *Aphis fabae* has an AAP of 60 min, and its optimum AAP is reported to be 12-16 hours (Roberts, 1940), while *Myzus persicae* has an AAP of 5 - 120 min with an optimum of 60 min (Sigiura and Bandaranayake, 1975). Sigiura and Bandaranayake (1975) further indicate that AAP varies according to the hosts involved.

Aphids, whiteflies and thrips reported above also occur in Uganda (Baliddawa, 1990). Being that they transmit tomato viruses to crops in a range of other countries (Brunt *et al*., 1990), a similar situation is expected in Uganda. However, vector seasonality and ability to spread tomato virus diseases in Uganda, in space and time, is not known (Legg, 1996). This absence of data has affected development of appropriate management strategies for tomato viruses in Uganda (Varela, 1995).

2.1.3.1.1 Virus Transmission by Whitefly Vectors

Whiteflies are vectors of viruses causing many diseases in the tropics and subtropics (Duffus, 1992). Bohmfalk *et al*. (2006) described the whitefly as a snowwhite insect measuring about 1mm in length. The adult whitefly starts laying eggs immediately after emerging from the nymph. Eggs are laid underneath leaves to protect them from adverse weather conditions such as rainfall and direct solar radiation (Marks, 2006). The adult is able to lay eggs for 3 weeks. Eggs hatch into nymphs, which measure about 0.5mm in length. Nymphs start by crawling about for short distances, but come immobile when they start feeding on the host leaf. Nymphs feed for 5-6 days, and after another 5-6 days, they go into pseudo-pupal stage for 2 days, after which they become adult flying whiteflies (Figures 2.2). Whitefly species *Bemisia tabaci* and *B. argentifolii* (Cohen *et al.*, 1992; Bellows and Perring, 1994; Riley *et al*., 1995; Legg, 1996), as well as *Trialeurodes vaporariorum* and *T. abutilonea* (Larsen and Kim, 1985) transmit geminiviruses. Among these there many now known biotypes (Demichelis *et al*., 2000). There is variation between whitefly biotypes in the ability to colonize plants and transmit geminiviruses (Rataul and Brar, 1989; Legg, 1996). Whiteflies colonize plants by laying their eggs underneath leaves, where they hatch into nymphs. Ugandan cotton and sweet potato whitefly biotype strains are said not to reproduce on cassava (Legg, 1996). Neither their adults nor their nymphs survive on cassava unlike the Ivory Coast strain, but both their adults and nymphs survive on cotton (Legg, 1996). *B. tabaci* also infests other hosts such as *Commelina benghalensis* L. and *Euphorbia heterophylla* L. (researcher's field observation). These plants are weeds found growing along with tomato, tobacco, cucurbits, and many other tropical crops. In spite of these efforts, *B. tabaci* ability to colonize tomato, tobacco and cucurbits in Uganda is not yet well known (Legg, 1996).

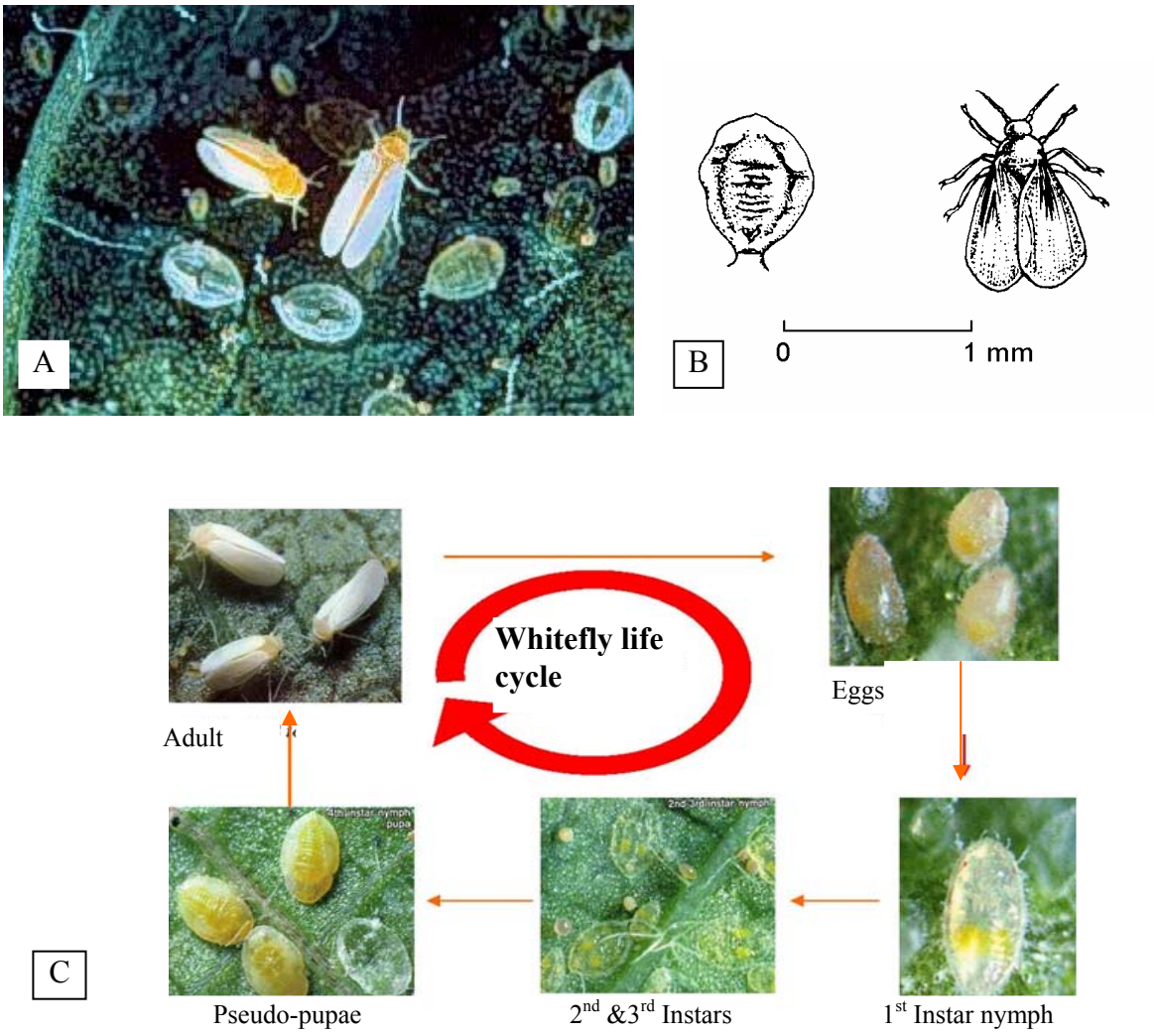


Figure 2.2: Whitefly vector (*Bemisia tabaci*): A- Winged adults and nymphs, B- Sketch drawings to give an impression of actual size of the adult whitefly and nymph (www.bijlmakers.com/guide), C- Whitefly Life cycle (www. 2.dpi.qld.gov.au/image)

Whitefly vectors (*B. tabaci*) are phloem feeders and transmit tomato yellow curl viruses from chilli to tomato and vice versa (Rataul and Brar 1989; Jiang *et al*., 2000). However, according to Caciagli *et al.* (1995) transmission is only possible from tomato to tomato. Caciagli *et al.* (1995) and Rataul and Brar (1989) found that virus acquisition, referred to as AAP (access and acquisition period, or access to plant cells and sucking sap from it to acquire virus inoculum), takes 31 min to 24 hours, while virus inoculation, IAP

(inoculum access period, or the period during which a vector pierces cells and infects plant with virus inoculum), takes 32 min to 24 hours. In contrast, Ber *et al.* (1990), reported an AAP of 48 hours, and that infected plants take a maximum of 15 - 29 days to show symptoms. However, it was found that there is a need for a pre-and post-acquisition fasting period of at least 1 - 2 hours to enhance transmission efficiency. Cohen and Nitzany (1966) reported a latent period of 21 hours before the vector transmits the virus. Caciagli *et al.* (1995) found that the latent period is 17 - 20 days. They also established that the virus persists in the vector for 20 days. These scientists seem to agree that whitefly transmission is persistent, i.e. able to infect plants with virus inoculum all the time, whereas inoculum is not carried over to their offsprings.

Furthermore, Caciagli *et al.* (1995) found that virus acquisition is more efficient than inoculation, and females are more efficient in transmission than males, while nymphs are as efficient as adults in acquiring the virus, but of little epidemiological importance because of being immobile.

Rataul and Brar (1989) determined transmission efficiency by use of χ^2 analysis of data from number of plants infected and expected number of plants to be infected. Other methods of analysing transmission are through calculating the probability of disease transmission (Rataul and Brar, 1989) by:

$$p = 1\text{-}Q^{1}/k \qquad (5),$$

whereby p is probability of disease transmission by single vector, Q is observed fraction of non-infected plants; and k is number of insects used per plant.

Furthermore, the effect of IAP on transmission by a single vector is determined by expressing the growth rate of increase in infection, which is expressed as (dp/dt), and is said to increase linearly with the proportion of uninfective insects.

2.1.3.1.1.1 Whitefly Vector Population in the Agro-ecosystem

In many parts of Africa, seasonal contrasts in rainfall influence pest populations (Elkinton, 1993). Rainfall provides adequate moisture for growth of plant hosts, but also affects the success or failure of oviposition and egg development of the vector (Duffus, 1992).

Nono Womdim *et al.* (1996) reported that in Tanzania, whitefly population size changed with season, the major variables being rainfall and temperature. Similar results were reported for Egypt (Moustafa, 1991). Riley and Wolfenbarger (1993) recorded a similar pattern in *Bemisia tabaci* populations, which change from year to year and season to season. Natural enemies and pesticides were also found to affect whitefly populations. Riley *et al*. (1995) reported that natural enemies and agricultural pesticides, in addition to climatic factors influence whitefly population dynamics. Furthermore, Baumgartner and Yano (1990) stressed the role of natural enemies. It was established that the use of pesticides reduces populations of natural enemies, and subsequently enhances whitefly population growth, while the possibility for whitefly resistance to pesticides was not ruled out (Duffus, 1995). Another factor influencing the development of whitefly vector populations is intensified agricultural practices.

In the Americas, international transport of plant materials introduced new *B. tabaci* biotypes into the ecosystem (Duffus, 1992; Polston and Anderson, 1997). On the other hand, plant hosts already in the ecosystem do also affect whitefly population dynamics. Legg (1996), while working with whiteflies on cassava, found that *B. tabaci* population growth on cassava, sweet potato and cotton host plants differed from each other. He observed that there were variable establishment success levels on individual crops. *B. tabaci* survived better on cotton than on any other host (Legg, 1996; Byrne *et al*., 1990; Von Arx *et al*., 1983). Lettuce (*Lactuca sativa*) was also reported to be a very good host for *B. tabaci* (Byrne *et al.*, 1990). It was further observed that there were variable establishment success levels on individual crops. It was further observed that as whiteflies migrate from one host to another, their population increases on the new host as

it decreases on the old host (Byrne and Bellows, 1991; Butler and Henneberry, 1989). Therefore, whitefly vector population growth cannot be restricted to any single pattern, since climatic conditions, types of plant hosts, and levels of pesticide use are highly variable from one agro-ecosystem to another. Based on this experience, Riley and Wolfenbarger (1993) suggested that, in order to understand *Bemisia* spp. population dynamics, there is a need to go beyond the micro-level scale to the macro-level, which implies a system wide approach to understanding whitefly population dynamics. It is also very important as a basis for developing local and regional population dynamics models (Allen *et al.,* 1994; Anderson, 1992; Bellows and Arakawa, 1986; Cohen *et al.*, 1988).

To study whitefly population dynamics, sampling tools have been developed with time. Gerling and Mayer (1995) indicated that the only way to determine *Bemisia* spp. population dynamics is by use of relative measures or estimates as opposed to direct insect population determination. An example of relative population figures is the number of whiteflies trapped by sticky traps in a field as opposed to counts of adult whiteflies or nymphs, which gives direct measures of the whitefly population per plant. Absolute measurement of *Bemisia* spp. field population is difficult to perform, because whiteflies react very fast to disturbances in their environment, and fly away. This makes it difficult to count them. Therefore, Ohnesonge and Rapp (1986) established that sticky traps could be used as relative measures to monitor adult whitefly population changes. Ohnesonge and Rapp (1986) and Cohen *et al.* (1988) further observed that while sticky traps are useful for assessing adult populations, these techniques could also measure migrating whiteflies, and even those making trivial flights within the field, and on individual plants. However, according to Butler *et al.* (1989), direct measures of adult whitefly populations can be taken with sticky traps, or by turning over the leaf to enable direct counts, or by other insect trapping methods such as use of Sunmica plate, vacuum collector, and passive fan trap. It was found that for nymphs, counts made after turning over the leaf is the best sampling tool, as they do not move around. For Butler *et al.* (1989) indirect measurements of whitefly population dynamics were by scoring intensity of sooty mould, different forms of crop damage, and virus transmission as proxy.

Although the above methods are sometimes used, there are a number of disadvantages associated with them. For example, turning leaves and taking leaf sample measures gives an estimate of whiteflies on the plant, but these methods are very tedious and inefficient because adult whiteflies fly away upon turning the leaf and they seldom oviposit on tomato, in which case nymphs are not even available for counting. Consequently, there is a need to develop a more efficient sampling tool that would be able to provide whitefly population data for single tomato plants in the field, as was provided for in this study.

2.1.4 Principles of Tomato Virus Disease Management

According to Palumbo *et al*. (2001), cultural and biological pest management tactics provide the best options for controlling *B. tabaci* and overcoming the problem of insecticide resistance. As such, various cost effective measures have been reported for controlling tomato virus diseases. They include cultural practices, vector manipulation, inoculum source elimination or phytosanitation, cross-protection, use of resistant varieties or even transgenic plants, and virus or vector exclusion (Matthews, 1991; Dent, 1991; Beachy, 1997; Mason *et al*., 2000; Hilje *et al*., 2001; Lapidot and Friedmann, 2002; Greer and Dole, 2003; Rubio *et al*., 2003; Yang *et al*., 2004; Mutwiwa *et al*., 2005; de Castro, *et al*., 2005; Kumar *et al*., 2006; Zilahi-Balogh *et al*., 2006; and Fuentes *et al*., 2006).

2.1.4.1 Integrated Vector Manipulation (IVM)

IVM is the application of a number of vector control options at the same time. IVM plays a significant role in control of many viral diseases. Previous reports (Simons *et al.*, 1959; Simons *et al.*, 1980; Palumbo *et al*., 2001; Greer and Dole, 2003) indicated that use of either pesticides, or mineral oils, barriers, insect traps, reflective mulches such as white polythene and aluminium foils, insect parasites, predators, and male sterility led to management of insect pests such as aphids, thrips, and whiteflies, which are also virus vectors. There are also biological control options, though they are mostly used in screen houses. Whiteflies are parasitised by several wasps such as *Encarsia formosa* and

Eretmocerus spp., as well as predators like lacewing and coccinelids (Zalon *et al*., 2003). However, concentrating on vector control is not always very effective since some vectors transmit viruses in a persistent manner (Green, 1991; Gianessi *et al*., 2003), so that even at very low densities, some vectors are effective in transmitting viruses. Moreover, transmission of non-persistent viruses takes place quickly and readily, and is often performed by vectors that visit, but do not establish their colonies on the visited crop. According to Perring *et al*. (1999), no one method of control is likely to keep crops free of vectors and virus infections.

2.1.4.1.1 Crop Sanitation

A number of cultural practices have been reported to reduce incidence and severity of viral diseases. These include elimination of inoculum sources by removal of infected plants and alternative hosts. Chan and Jeger (1994), while working with an analytical model of plant virus disease dynamics with uprooting and replanting, found that in sparsely planted crops intensive uprooting only was able to eradicate virus diseases. Cho *et al.* (1989) reported that removing TSWV-infected crop plants, weeds and alternative hosts, in an overall integrated management package, helped to control viral diseases. Lloyd *et al.* (1974) were able to eliminate banana viruses by use of virus-free planting material obtained by thermal therapy and tissue culture. Other effective methods reported are debris removal from the field, disinfection of support trellises before re-use, crop quarantine measures executed for incoming seeds, and crop rotation (AVRDC, 1985; Green and Kim, 1991); use of skimmed milk foliar sprays (Black *et al.*, 1991; Green *et al*., 1991); application of antiviral agents like cytovirin (Simmons 1959), and treatment of tomato seed with trisodium phosphate for 1 hour (Broadbent, 1965 and 1976).

2.1.4.1.2 Cultural Control

There are a number of cultural control measures, such as close plant spacing to compensate for yield losses through diseased plants, use of mulching and intercropping with nitrogen fixing cover crops like *Lablab purpureus* L. (Cohen *et al.*, 1974). Greer and

Dole (2003) reported that aluminium foil and mulches are effective in repelling insect pests, and that black plastic mulches improve crop yield better than bare ground. Mutwiwa *et al*. (2005) found that ultra-violet light absorbing plastics used as mulches or green house roofing material repel whitefly pests when low UV-intensity plastics are used. Other cultural options include timely planting, intercropping, and use of barrier hedges (Simons *et al*., 1980). These methods effectively reduce tomato yield losses, virus incidence and spread, even though they are simple and common.

2.1.4.2 Cross-protection

Cross-protection is the method of controlling virus disease by using a mild virus strain to immunize otherwise healthy plants against a severe virus strain (Matthews, 1991). The method has been used against ToMV and TSWV. Avgelis (1987) used a mild strain (MX lV – l) of ToMV to cross-protect ToMV-susceptible tomato cultivars Earlypak and Dombo C2 VF2 in Crete, leading to 14% yield improvement. He expected higher yields, and as such attributed low increase in yield to higher than expected temperatures, which could have reduced the effectiveness of mild strains. Another example of cross-protection is when Min-Wang and Gonsalves (1992) and Cho *et al.* (1989) used nitrous acid to induce mutation of TSWV to mutant R27G, which was put in tomato in order to control severe TSWV strains from Hawai and Oklahoma.

This method requires more sophisticated facilities and careful management of the outcome, which could otherwise result into more dangerous strains. With limited resources in the south, one would to be very careful to recommend use of such a technology, even though it could be required in some instances.

2.1.4.3 Host Plant Resistance

Use of resistant crop varieties is the most convenient and cost-effective control measure of all (Hall, 1980; Gajos, 1981; Kumar and Irulappan, 1992; Nono-Womdim, 1993; Rubio *et al*., 2003; Yang *et al*., 2004; de Castro *et al*., 2005). For example, planting of

tomato variety Pearl Harbour (Yassin and Nour, 1965) provided a tomato crop with resistance to *Tomato yellow leaf curl virus* (*sensu stricto*). Twelve tomato lines from Israel were found to be partially resistant to TYLCV (de Catro *et al*., 2005). Krishna-Kumar *et al.*(1995) found resistance to TSWV in wild tomato species on the Hawaiian Island of Maui. Legnani (1995) reported resistance to PVY in wild tomato (*Lycopersicon hirsutum*), which was subsequently used in breeding programmes. Moreover, some hairy wild tomato species are said to be resistant to CMV (AVRDC, 1985).

Such resistance to CMV has been identified in another wild tomato, i.e. *Lycopersicon chilense*, which has also got resistance to TYLCV (Zakay *et al.*, 1991). In Thailand, the most tolerant lines of tomato to TYLCV are Fl (106) 1 (33) (21) Fireball and P1 (30) 5 (29) (16) P-1. To develop virus-resistant tomato varieties, it is necessary to understand mechanisms involved. Bejarano and Lichtenstein (1994) reported about engineering tomatoes with resistance to TYLCV. According to Aranyine-Rehak and Burgyan (1992), pseudo-recombinants, which are RNA strands forming loose bonds with complementary target virus RNA strands, were used for specific determination of virus genome sections responsible for specific functions. In their report, pseudo recombinants of *Tomato aspermy virus* (TAV) and *Cucumber mosaic virus* (CMV) were used to determine RNA responsible for cross-resistance in CMV. As a result, RNA-1 and RNA-2 were found to be responsible for resistance to CMV strain (CMV nt 80/35), which induces intense yellow mosaic symptoms. Also the use of transgenic plants for resistance to TYLCV, a method that is encouraged as the easiest way to manage TYLCV in the future (Beachy, 1997), has been researched. Bendahmane and Gronenbom (1997) used anti-sense RNA, which targeted mRNA of the replication gene (C1), to engineer resistance against TYLCV. Using *N. benthamiana* test plants, they found that TYLCV could be resisted. At least 21% of tested plants were found resistant to TYLCV when Antignus *et al*. (2004) used truncated replication associated protein (T-Rep) gene from a mild TYLCV strain. Just recently, Fuentes *et al*. (2006), reported transgenic tomato plants, which were transformed with an intron-hairpin genetic construction (726 nt of the 3' end of TYLCV C1 gene) to induce post transcriptional gene silencing against early TYLCV replication

gene (C1). This line of castor bean catalase intron-hairpin transgenic tomato plants was resistant even at high whitefly populations. These results exhibited a new trend of other possible management solutions for TYLCV (*sensu lato*), which are worth trying in developing countries like Uganda.

Whereas many different control methods have been developed for tomato viruses elsewhere, none of them have been tested in Uganda. This study, therefore, is seeking to (1) identify tomato viruses in major tomato growing areas of Uganda; (2) generate information on one virus found to be a major virus problem on tomato; (3) establish the epidemiology of this virus in the complex small holder agro-ecosystem; and (4) investigate its relationship with the vector. Such information could eventually be utilized in the development of integrated viral disease management packages, such as transgenic plant resistance.

CHAPTER 3

A SURVEY OF TOMATO VIRUSES IN MAJOR TOMATO GROWING DISTRICTS OF UGANDA: VIRUS INCIDENCE, DISTRIBUTION AND IDENTIFICATION

In this chapter, the objective is to identify and establish incidence of tomato viruses occurring in major tomato growing agro-climatic zones of Uganda

3 CHAPTER 3

A Survey of Tomato Viruses In Uganda: Incidence, Distribution, and Identification

3.1 Introduction

It is known that 146 tomato viruses exist worldwide[1]. According to available reports, some of these viruses occur in East Africa. As early as the 1960s, reports from neighbouring Tanzania indicated occurrence of *Tomato spotted wilt virus* (TSWV) (CMI, 1969), *Tomato yellow leaf curl virus* (*sensu stricto*) and *Tomato leaf curl virus* (ToLCV-TZ), *Tomato mosaic virus* (ToMV), *Cucumber mosaic virus* (CMV), and *Potato Virus Y* (PVY) (Nono-Womdim *et al*., 1996; Chiang *et al*., 1996). *Tomato yellow leaf curl virus* (TYLCV) (*sensu stricto*) and *Tomato leaf curl virus* (TLCV-SD) (Yassin, 1989; Brunt *et al*., 1990) have also been reported from Sudan. However, *Potato Virus X* (PVX) and *Chili veinal mottle virus* (ChiVMV) have not been reported on tomato from the East African region, and the report available to me about viruses occurring worldwide (Brunt *et al*., 1990) has nothing about PVX or ChiVMV occurrence on tomato in this region.

In Uganda, there is lack of information on prevailing tomato viruses. However, the occurrence of some tomato viruses in neighbouring countries, together with poor quarantine measures, and the ability of vectors to move across borders, implies a high probability of Uganda having similar tomato viruses. The actual information gap on tomato viruses occurring in Uganda is basically due to attribution of tomato crop losses caused by viral diseases to other production constraints (Akemo and Ssekyewa, 1996 unpublished work on general tomato agronomy in report submitted to IPM-CRSP Project). As such, and apart from the general survey on horticultural crop viruses conducted by Hansen (1990), during which mosaic, curl and mottle symptoms were observed in tomato farmers' fields, there was no other more specific report on tomato

[1] http://image.fs.uidaho.edu/vide/descr002.htm

virus diseases in Uganda. Where more detailed and correct viral diseases information is available, tomato leaf curl viruses alone are reported to cause 100% crop yield loss (Czosnek and Laterrot, 1997). This devastating situation could also be possible for Uganda.

Major tomato growing areas in Uganda have dry and wet seasons alternating in a year (Mukiibi, 2001; Ministry of Agriculture Zoning Report, 2005 unpublished, Annex 1). The dry seasons are between June to August and January to February, while wet seasons are between March to Mid-June and Mid-August to December. Elsewhere, seasonality is known to affect tomato virus diseases incidence and distribution (Moustafa, 1991). Therefore, our objective is to survey for incidence and distribution, and to identify viruses infecting tomato in Uganda, as was done elsewhere (Czosnek and Laterrot, 1997; Green *et a*l., 1991; Bock, 1982; Martyn, 1968). We assume that viruses occurring on tomato in neighbouring countries also infect tomato in Uganda. Knowledge of these viruses would provide a sound basis for future resistant tomato variety introduction and breeding programmes.

3.2 Materials and Methods

3.2.1 Field Survey

During the first rainy season (March-June of 1997), second rainy season (August-November 1997, the dry season (January-February 1998), and the first rainy season (March to July 1998), a survey was conducted to cover major tomato growing districts in Uganda, i.e., Iganga, Kasese, Kabale, Mbale, Mbarara, Mpigi, Mukono, and Rakai (Figure 3.1). According to the Ministry of Agriculture, Animal Industry and Fisheries (MAAIF) and the National Agricultural Research Organization (NARO), these districts are located in five agro-climatic zones (Table 3.1 and Annex 1), which are among the ten zones into which Uganda is divided (Annex 1).

Based on NARO and MAAIF zones, a survey was conducted using a random stratified design (Elkinton, 1993). Zones and districts formed the first stratum of sampling, while farmer fields were randomly selected using calculator random numbers to form the second level of sampling. Five tomato fields (of about 0.25 – 1 ha each) were randomly selected in each district.

3.2.1.1 Collection of Virus Symptoms-bearing Tomato Samples

For each of the five fields in every selected district, twenty leafy shoot samples were picked from individual plants showing virus-like symptoms by the smart-sampling approach (Bragard, 2006 personal communication), and were placed in paper bags. At least five leaf curl samples were collected from each district, except for Kasese, where neither leaf curl nor mottling symptoms were observed during the visit made in the dry season (January-February 1998) until later at the beginning of the rain season (March-July 1998), probably because inoculum-bearing plants had died and vector population decreased to very low levels at the end of the previous growing season, a situation referred to as random extinction[2] by Garcia-Arenal *et al*. (2000). Ohnesonge *et al*. (1981) report about death of whiteflies during the winter season, which is another good example. In addition to the 800 leafy tomato shoot samples, suspected weed samples were also collected from within and around tomato fields to determine tomato viruses host range (Bock, 1982). Detailed field notes were made on virus symptoms, presence of vectors such as aphids by simple observation, and varieties grown as well as general cropping practices such as intercropping by interviewing farmers (Annex 3). Vector infestations of tomato fields were recorded as low, medium, or high, if (≤25%), (25% < to 50%), and (50% < to 100%) of tomato plants in the field were infested, respectively (Cho *et al.*, 1989). Virus symptom severity was scored on a scale of 1-5, based on extent of leaf damage and percentage number of leaves showing symptoms, whereby 1=1-20% (very mild); 2=21-40% (mild); 3=41-60% (severe); 4=61-80% (very severe); and 5=81-100% (almost dead). Virus disease incidence, defined as the extent of infection in the field, and calculated according to Allen *et al*. (1983) formula:

[2] In this report by Garcia *et al*., 2000, the world random seems to be used to mean sudden

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Number of plants in the field}} \times 100 \quad (6),$$

was recorded and estimated as percentage infection, whereby 1-20% =low incidence; 21-49% = moderate incidence; and 50 - 100% = high incidence (Nono-Womdim *et al.*, 1996).

In the laboratory, samples were first grouped according to symptoms, which included mosaic, mottling and leaf curl (Figures 3.3, 3.4, and 3.5). Those samples with no definite symptoms were grouped under 'miscellaneous' (Figure 3.6), as compared to healthy plant leaves (Figure 3.2). Because tomato yellow leaf curl is reported to be caused by more than one virus species (Czosnek and Laterrot, 1997) and the causal agent was earlier on demonstrated to be a geminivirus (Cohen and Harpaz, 1964) with no known efficient antisera, each sample that expressed tomato leaf curl symptoms was also squash-blotted onto High Bond Nylon membrane as described by Czosnek *et al*. (1988); Czosnek *et al.* (1990) and Green and Kalloo (1994). Squash-blotted Nylon membranes were later taken to the Laboratory of Professor. D. Maxwell, University of Wisconsin-Madison, USA, for DNA hybridisation. Some of these samples were treated and dried like any other in preparation for later Polymerase Chain Reaction (Czosnek *et al*., 1988) studies. Some other collected samples were chopped into small pieces, prior to sample analysis. After chopping each sample, the knife was dipped into ethanol and heated over a spirit burner flame to avoid contaminating subsequent samples. Leaf pieces were then put on a filter paper and sealed in petridishes over anhydrous calcium chloride to dry at 4ºC (Green, 1991), and were kept in those conditions for 5-9 months. Later on, each sample was put in a labelled self-sealing polythene bag over anhydrous calcium chloride for easy transportation to the World Vegetable Centre (AVRDC) Associated Laboratory at Horti-Tengeru, Arusha, Tanzania, specifically to conduct DAS-ELISA tests that could not be done in Uganda at the time.

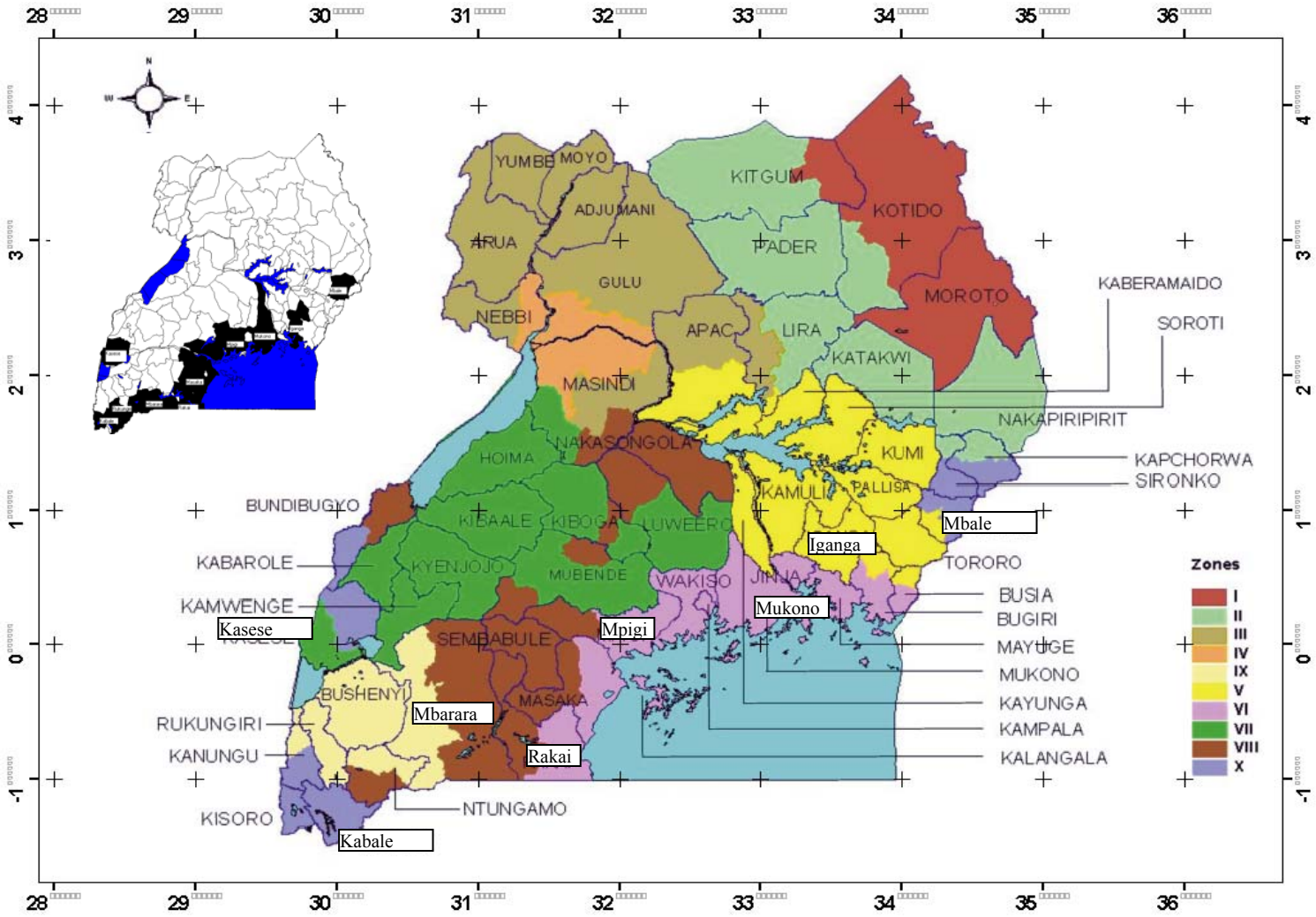
Table 3.1: Agro-climatic zones of Uganda and major characteristics of production systems (NARO, 1999). Surveyed districts are underlined.

Agro-ecological zone	Production system/Districts	Major traditional cash and food crops	Production constraints and challenges (NARO, 1999)
Long Rain Unimodal	Northern: Gulu and Kitgum	Cotton, tobacco, sesame, sorghum, cassava, groundnuts and finger millet.	Poor road and storage infrastructure and distance to markets
Transitional	Eastern: Katakwi, Kumi, Soroti, Tororo, and Pallisa	Cotton, finger millet, groundnuts, cassava, rice, sweet potato, and cowpeas	Declining soil fertility and unreliable rainfall
	Mid-Northern: Apac and Lira	Cotton, sesame, sorghum, cassava, pigeon peas, beans, sunflower	Declining soil fertility and unreliable rainfall
	Lake Albert Crescent: Masindi, Hoima, Kibaale.	Cotton, bananas, finger millet, cassava, maize beans, groundnuts, citrus	Declining soil productivity
	South-Eastern: Iganga, Bugiri, Busia, Kamuli, Jjinja.		
Bimodal rainfall	Lake Victoria Crescent: Mukono, Mpigi, Masaka, Nakasongola, Luwero, Kiboga, Mubende, Rakai	Robusta coffee, banana, maize, tea, vanilla, beans, horticultural crops	Pests and Diseases
Montane	Eastern Highlands: Mbale and Kapchorwa	Maize, wheat, arabica coffee, banana, finger millet, beans	Population pressure and declining soil productivity
	Southernhighlands: Kabale, Kisoro and Rukungiri	Irish potatoes, sorghum, finger millet, peas and onions	Population pressure and declining soil productivity
	Western highlands: Kasese, Bundibudyo, Kabarole and Bushenyi	Banana, cassava, sweet potato, finger millet, beans, maize, arabica coffee, cotton and passion fruits	Inadequate infrastructure and access to market
Dryland	Karamoja: Kotido and Moroto	Sorghum, cowpeas, pearl millet and finger millet	Overstocking and environmental degradation
	Southern: Mbarara, Sembabule, Ntungamo, and Rakai	Beans, finger millet, and banana	Overstocking and environmental degradation

Figure 3.1: Districts of Uganda and Ministry of Agriculture Animal Industry and Fisheries (MAAIF) zoning (I-X) (MAAIF)

Naming of zones (See Annex 3 for detailed description):
I – North Eastern Dry lands
II – North Eastern Savannah Grasslands
III – North Western Savannah Grasslands
IV – Para Savannah
V – Kyoga Plains
VI - Lake Victoria Cresent
VII – Western Savannah Grasslands
VIII – Pastoral Rangelands
IX – Southern Western Farmlands
X – Highland Ranges

High lighted in a box is each district that was surveyed, and in set is a map of Uganda showing survey areas as black shaded parts in five agro-climatic zones, i.e. Highland ranges (X), Kyoga plains (V), Lake Victoria Cresent (VI), Western Savannah Grasslands (VII), and Southern Western Farmlands (IX).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ARUA
GULU
NEBBI
KITGUM
KOTIDO
PADER
MOROTO
APAC
LIRA
KABERAMAIDO
SOROTI
KATAKWI
MASINDI
NAKAPIRIPIRIT
NAKASONGOLA
HOIMA
KUMI
KAPCHORWA
SIRONKO
KAMULI
PALLISA
BUNDIBUGYO
KIBOGA
LUWEERO
Mbale
Iganga
KABAROLE
KYENJOJO
MUBENDE
WAKISO
JINJA
TORORO
KAMWENGE
Kasese
Mpigi
Mukono
BUSIA
BUGIRI
MAYUGE
SEMBABULE
MUKONO
KAYUNGA
BUSHENYI
MASAKA
Mbarara
RUKUNGIRI
KAMPALA
KANUNGU
Rakai
KALANGALA
KISORO
NTUNGAMO
Kabale
Zones
I
II
III
IV
IX
V
VI
VII
VIII
X

Figure 3. 2: Healthy tomato plants: (A) leaves show some green leaf curling which is normally due to uptake of excess water (turgidity of leaf cells) for a luxuriantly growing tomato on fertile soils rich in nitrogen. Leaves in (B) are characteristic of a normal healthy tomato plant at flowering stage.

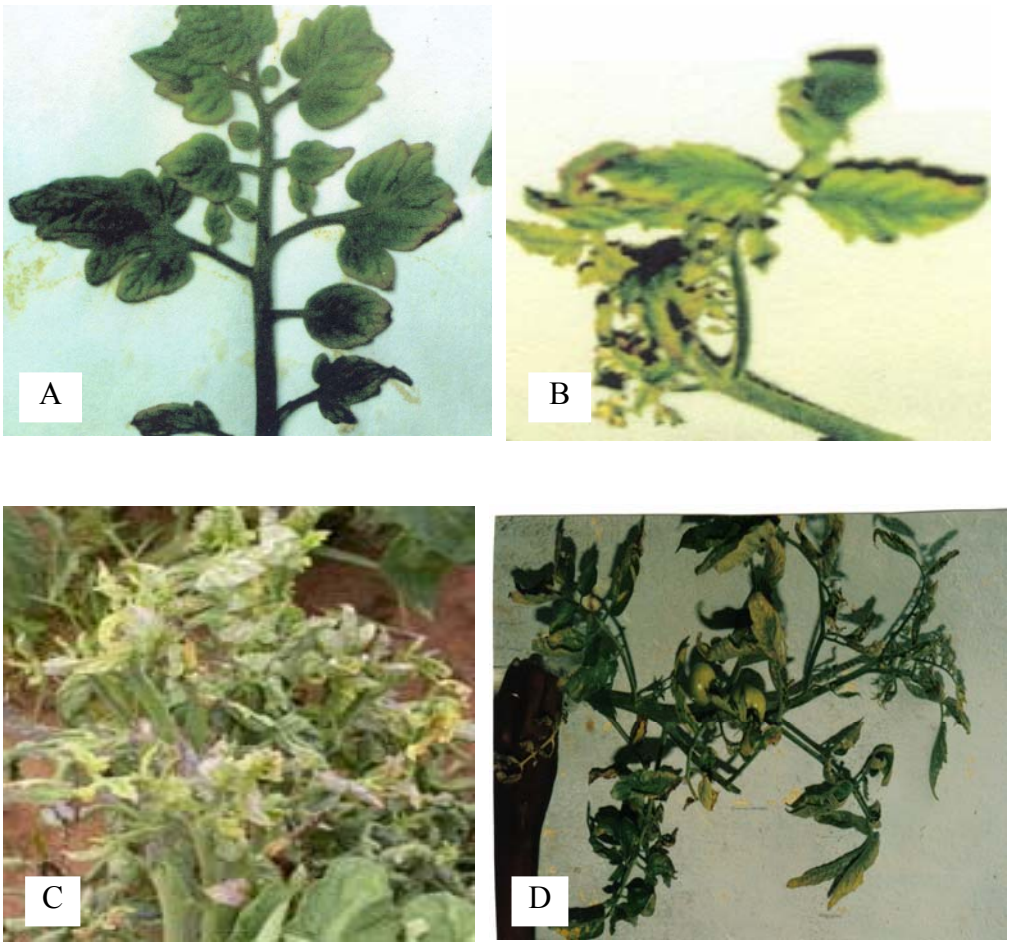


Figure 3.3: Tomato leaf curl symptoms as observed in fields surveyed in Uganda: A- roundish leaves, B- marginal yellowing, C- Severely curled tomato shoot tips (AVRDC) similar to our own observation, D- One of our positive samples with mild secondary infection leaf curl symptoms and only one trush of fruits.

Figure 3.4) Systemic green mosaic symptoms on tomato leaves positive to (A) *Pepper veinal mottle virus* (PVMV) infected plant, which tested positive to PVMV antiserum (SANOFI); (B) single leaf from the same plant with green mosaic symptoms

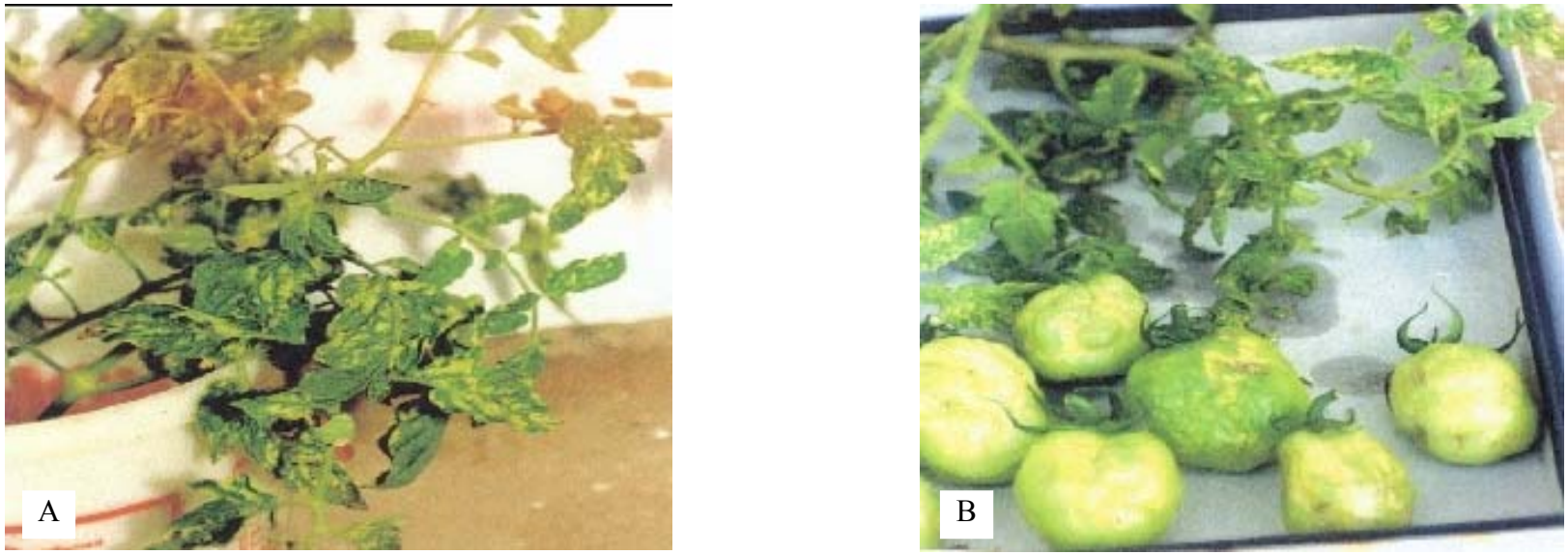


Figure 3.5 *Alfalfa mosaic virus* (AMV): (A) yellow mosaic leaf symptoms; and (B) severely distorted tomato fruits from an infected plant. The sample was collected from Kasese Irrigation scheme, where many plants expressed the same symptom and tested positive to AMV, with AMV antiserum (SANOFI)

Figure 3. 6: Some miscellaneous symptoms observed on tomato plants in the surveyed area. Some of these (A, E, F) seem to be due to the plant's reaction to pesticides

3.2.2 Identification of Viral Diseases

3.2.2.1 Serological Identification of Tomato Viruses

Double Antigen Sandwich-Enzyme Linked Immunosorbent Assay (DAS-ELISA), as described by Clark and Adams (1977), was selected because it is efficient and cheap, and after all Nono-Womdim *et al.* (1996) used it successfully to identify tomato viruses in Tanzania. Therefore, we used DAS-ELISA to confirm that symptoms seen in the field were actually due to virus causal organisms. The technique was also used to identify those viruses infecting collected tomato leafy symptom-bearing samples. Mottling and mosaic symptom-bearing tomato leaf samples, which were collected, dried and preserved over calcium chloride, were then packed in self-sealing polythene bags, with five grams per bag. Dry samples were then crushed to powder. A total of 10 ml of extraction buffer (0.03 M phosphate buffer, pH 7.4) was added to each bag. The mixture was ground with a pestle to extract sap, which was used for ELISA tests (Bar-Joseph and Hull, 1974). Using DAS-ELISA, mottling and mosaic symptoms-bearing tomato leaf sample extracts were tested for eight viruses commonly encountered in Africa (Brunt *et al*., 1990; Nono-Womdim *et al*., 1996) and for which antisera could be obtained commercially. These were ToMV, TSWV, PVMV, PVY, PVX, CMV, AMV and ChiVMV. Polyclonal antisera and conjugates for these viruses were obtained from SANOFI, France.

Based on standard dilution recommendations, polystyrene microtiter plates were coated with immunoglobulins (IgG) of the eight viruses at the following manufacturer's given concentrations; 30µl/15ml (ToMV), 300µl/15ml (PVMV), 30µl/15ml (TSWV), 60µl/15ml (PVY), 150µl/15ml (PVX), 150µl/15ml (CMV), 30µl/15ml (AMV) and 150µl/15ml (ChiVMV) in sodium carbonate coating buffer (0.2M, pH 9.6), and incubated for 3 hours at 37 ºC to achieve maximum detection of target viruses. One hundred microlitres of coating buffer and immunoglobulins solution was put in each well. Plates were then washed three times with 0.01 M phosphate-buffered saline (PBS-Tween 20) buffer, and dried over tissue paper (Nono-Womdim and Atibalentja, 1993). Plate wells were filled with 0.10 ml of tomato sample extract ground in PBS pH 7.4 (1:2, W/V) and

incubated at 4ºC overnight. After rinsing off excess antigens, 0.10 ml of conjugated IgGs were added per well and incubated at 37ºC for 3 hours. Plates were again washed with PBS-Tween 20 and dried before adding 0.10 ml substrate p-nitrophenyl phosphate (1mg/ml) in 0.1 M diethanolamine substrate buffer at pH 9.8 (Clark and Adams, 1977).

Microtiter plates, which had the substrate added, were incubated for 1-2 hours at room temperature in a dark place to reduce background reaction. Background reaction refers to exposure of loaded microtiter plates to excessive light causing very fast reactions, which cause yellowing of empty wells, hence masking positive sample well reaction. Positive samples were recognized from wells that turned yellow, while healthy control remained colourless (Nono-Womdim *et al.*, 1996). Plates were also read with a MULTISKAN MS, Primary EIA 1.5-0 plate reader with a 405 nm absorbency filter. A well reading was considered positive if its absorbency value was three times that of a healthy sample well. Blanks should have absorbency values of less than 0.1 (Nono-Womdim personal communication). Data generated on number of samples testing positive or negative were analysed for percentages and frequencies with MSTATC statistical programme.

3.2.2.2 Molecular Identification of Viruses

Molecular techniques were used to identify those viruses, which caused leaf curl in the field, but could not be detected in serological tests. Geminiviruses are some of the possible examples of such viruses (Anderson and Morales, 2005). One of the geminiviruses causing leaf curl is *Tomato yellow leaf curl virus* (TYLCV).

Tomato yellow leaf curl is one of the most devastating viral diseases of tomato caused by a number of virus species (Moriones and Navas-Castillo, 2000). These viruses could not be tested through DAS-ELISA with polyclonal antisera. Therefore, instead of using DAS-ELISA, molecular techniques like DNA hybridisation (Czosnek, *et al*., 1988) and polymerase chain reaction (PCR) (Nakhla and Maxwell, 1998; Navot *et al.*, 1992) were used. For PCR, TYLCV-specific and general primers were used as shown in Table 3.3, whereas for DNA hybridisation analysis samples were either dot-blotted or squash-

blotted onto nylon membranes to test for TYLCV DNA. For each sample squashed, a portion was dried over anhydrous calcium chloride at 4ºC, in petridishes (Green, 1991) and stored in the refrigerator.

Consequently, every yellow leaf curl symptom-bearing sample that did not test positive through DAS-ELISA or had very clear TYLCV associated symptoms and was straight away suspected to be caused by tomato yellow leaf curl viruses (*sensu lato*), was subjected to molecular tests (Table 3.2). Of 800 samples tested in serology, 102 tomato leaf samples were negative to any of the eight viruses. Tomato samples bearing leaf curl, mosaic and mottling, but not testing positive through DAS-ELISA included: sample MP4 with mosaic symptoms, MP11 with white mosaic symptoms, IG9 with green mosaic symptoms, IG8 with green mosaic symptoms, MB6 with bronzing, mild mosaic and curl symptoms, and sample MPz with severe mosaic symptoms. Some samples were tested more than once depending on their previous reactions.

3.2.2.2.1 Virus Characterization Using DNA Hybridisation

DNA hybridisation was used to test for the presence of TYLCV. It involved preparation of P^{32}-labeled probes and hybridisation of squash-blotted or dot-blotted nylon membranes (Czosnek *et al.*, 1988).

3.2.2.2.1.1 Sample Preparation for DNA Hybridisation

To prepare dot-blotted nylon membranes, preserved dry as well as fresh leaf curl and yellow mottling symptom bearing tomato leaf samples (Figure 3.3) were squashed in TE buffer (Tris-EDTA), with Kontes blue pestles in eppendorf tubes and placed on ice (Nahkla *et al*., 1993). Squashed samples were centrifuged for 5 min, at 5,000 rpm. Pellets formed were discarded and supernatant used for dot-blotting (Nahkla *et al*., 1993). QIAGEN Nylon Plus membranes from QIAGEN Inc., 28159 Av. Stanford Santa Clarita CA 91355, were used. For each sample, a dot-blot spot of 5 µl of supernatant was blotted twice on separate spots on the membrane to minimize errors. Blotted membranes were

dried on 3MM blotting paper (Czosnek *et al.*, 1988). Dot-blotted samples were CVMV, CVMVMxd, RL1, RL2, RL5, K1, K2, K3, IGl, MB8, KN, MB9, IG2, MB7, MB2, IG3, MB6, and ISOPOT. Two positive samples, i.e. TYLCV-EG and *Bean golden mosaic virus* (BGMV), obtained from Maxwell Laboratory, University of Wisconsin-Madison, were used as positive controls, while samples taken from a healthy tomato plant raised in our screen house, at Kawanda Agricultural Research Institute, was used for a negative control.

Out of 75 samples, which had clear leaf curl symptoms and were tested with DNA hybridisation, 15 were tested as dot-blots in TE buffer, and 60 were squash-blots. Each sample was tested against both general and specific probes. For the 15 dot-blots, Dellaporta DNA extraction had to be done (Dellaporta *et al*., 1983). These samples were then hybridised using a mixed probe of radioactive BGMV and TYLCV-EG probes. Membranes were exposed to two charged X-ray films for different durations, i.e. 6 hours exposure at 4 °C, overnight at -80 °C, or two days exposure respectively (Nahkla *et al*., 1993).

Preparation of P^{32}-labelled Probes

To prepare P^{32}-labelled probes, the PCR product of *Tomato yellow leaf curl virus* DNA, provided by Dr. Medhat Nahkla of Maxwell Laboratory, was thawed on ice and TE (Tris-EDTA) buffer added (Nahkla *et al*., 1993). It was then denaturated by heating in a water bath at 100 ºC for 2 min, and chilled rapidly on ice. Labelling buffer (5X), a mixture of dNTPs (dTTP, dATP, dGTP), a P^{32}-labelled dCTP, Klenow DNA polymerase I, and Nuclease-Free BSA were added together to a final volume of 50 µl.

Table 3.2 Some samples tested with PCR and DNA hybridisation virus identification techniques. Sample codes were derived from location names, suspected virus name, nature of plant/isolate, and date of collection. Symptom description is based on researcher's knowledge of symptom description and descriptions made by Brunt *et al*. (1990) and Jones *et al*. (1991) See also Annex 5.

Sample Code	Field Symptom Description
K1	Mosaic, leaf curl, small leaves, flower abscission
K3	Yellow mottle, flower abscission
MB6	Mottling
PVMV	Severe dark green mosaic
ISOPOT	Curl, small leaves
KN	Mosaic and chlorosis
RL2	Mottling, crinkling
RL5	Leaf curl, small leaves
K2	Leaf curl, marginal yellowing and necrosis, stunting, dieback and no fruits
MPz	Severe mottling
IG2	Leaf curl, marginal yellowing
IG3	Leaf curl, no marginal yellowing
IG1	Leaf curl
MB5/25/9/98	Mottling
MB6/25/9/98	Yellow mosaic and crinkling
MB1/25/9/98	Mottling
MB2/25/9/98	Leaf curl and stunting
MB3/25/9/98	Leaf mottling
MP11	Yellowish whitish mosaic
MB9	Yellow mosaic
MB7	Leaf curl, yellowing and bronzing
MB8	Severe crinkling, yellow mottling, small fruits

The reaction mixture was incubated for 1 hour at room temperature. The reaction was stopped by heating at 95 – 100 ºC for 2 min and quickly chilling on ice. A volume of 0.5 µl EDTA, 45 µl of water and 5 µl of dye (4% dextran blue, 0.2% orange G) was added and the mixture spun in a sephadex column. The spin-through was denaturated by adding 0.5M NaOH (0.15 volume of 2M NaOH), and was left to stand for 10 minutes (Nahkla *et al*., 1993).

Probe concentration was measured by use of a scintillation fluid, and scintillator readings were taken and multiplied by half the volume. Scintillation counter reading was 27 million (2.7 million counts per ml of hybridisation buffer) for the probe. This was above the minimum of 0.5-1 million counts per ml (Promega, 1993). Radio-labelled probes (P^{32})

used were *Bean golden mosaic virus* (BGMV) DNA as a general probe for geminiviruses (Gilbertson *et al.*, 1991) and TYLCV-EG DNA provided by Professor D.P Maxwell's laboratory (Nakhla *et al.*, 1993) as specific probe for TYLCV-Is (Padidam *et al.*, 1995).

3.2.2.2.2 Virus Characterization Using Polymerase Chain Reaction (PCR)

Samples that tested positive to DNA hybridisation were further analysed using polymerase chain reactions with various general and specific primers to determine presence and identity of geminiviruses (Navot *et al.*, 1992; Briddon and Markham, 1994; Wyatt and Brown, 1996).

3.2.2.2.2.1 Sample Preparation for Polymerase Chain Reaction

To extract DNA from samples bearing leaf curl and/or yellow mottling, a mini-preparation technique developed by Dellaporta *et al*. (1983) was used. A dry leaf disc sample, about 1 cm in diameter was used per eppendorf tube per sample for extraction of clean total DNA. The DNA was dried in a vacum centrifuge Speed-Vac® Savant, for 5-7 min to concentrate and dry it. The dry pellet was re-suspended in 100 µl of distilled water. In the case of dirty brown pellets, instead of re-suspending in water, the latter pellets were re-suspended in 500 µl of Dellaporta buffer and the whole extraction procedure was repeated to remove all salts that cause browning and hinder PCR reaction.

3.2.2.2.2.2 DNA Fragment Amplification

Extracted DNA, in a 50 µl reaction mixture (28 µl of double-distilled water, 5 µl of 10xdNTPs, 5 µl of 10x buffer, 5 µl of 10x MgC12, 0.2 µl of Taq DNA polymerase, 1µl of each primer, 5 µl of Dellaporta DNA and overlaid with 100 µl of mineral oil was amplified in a Pelkin-Elmer Thermocycler run at 94 ºC for 1 min, 55 ºC for 2 min, and 72 ºC for 2 min, for 30 cycles and 94 ºC for 1 min, 55 ºC for 2 min, and 72 ºC for 4 min, in one cycle. The reaction was then held at 18 ºC (Nakhla *et al*., 1998). By repeated

denaturation (94 ºC), hybridisation or annealing (55 ºC) and extension (72 ºC), target DNA fragments were amplified with their sizes doubling after each cycle.

Oligonucleotide primers used for general detection of tomato geminivirus were PAL1v 1978 and PAR1c 715, PAR Av 494 and PAR AC 1048 (Rojas *et al*., 1993; Nakhla *et al.*, 1993; Wyatt and Brown, 1996) as shown in Table 3.3. Specific primers used to test for TYLCV-Is were PTYCRv 21 and PTYC Rc 287; PTYC2v 1499 and PTYAL1c 2196; PTYV2v 466 and PTYC 2c 1814.

Table 3.3: Primers provided by Maxwell Laboratory, University of Wisconsin-Madison and those (PTLCV - UGrep2r, PTLCV - UGrep1f, PTLCV - UGcp 1f) produced during this study, and used in PCR reactions to identify and characterize geminiviruses

Primer Code[1]	5'Carbon end (Donor)–Sequence–3'Carbon end (Receptor) / DNA segment size	Nt annealing start-point
Degenerate		
PARAv 494	GCCCATGTATAGAAAGCCAAC	494
PARAc 1048	GGATTAGAGGCATGTGTGTACATG	1048
PAR1c 715	CATTTCTGCAGTTDATRTTYTCRTCCATCCA	715
PAL1v 1978	GCATCTGCAGGCCCACATYGTCTTYCCNGT	1950
Specific		
PTYCRv 21	AACTCTGCAGTTGAAATGAATCGGTGTCCC	2636
PTY IRc 287	ATATCTGCAGTTGCAAGACAAAAAACTTGGGGACC	123
PTYC 2v 1499	ATTTGTGGATCCTGATTACCTTCCTGATGTTGTGG	1499
PTYAL1c 2196	AAATCTGCAGATGAACTAGAAGAGTGGG	2196
PTYV 2v 466	TTAGGGATCCTATATCTGTTGTAAGGGC	466
PTYC 2c 1814	AAACGGATCCTTGAAAAATTGGGC	1814
PTLCVUGrep2r	GAGAATGTCATGAGTTCCGCTGCG	2078
PTLCVUGrep1f	GGGGATACCAGGTCGAAGATCGG	2437
PTLCVUGcp1f	GTATTACATAGGGTTGGCAAGAGG	641

[1] P (Primer); TY (specific to TYLCV); TLCVUG (specific to ToLCV-UG); IR (intergenic region); V1, V2, C1, C2, C3, C4 (different Open Reading Frames, as in figure 3.6 below); V (viral sense primer); C (complementary sense primer); f (forward); r (reverse); 21-2437nt (nucleotide numbers for TYLCV-Is specific primers and for BGMV-GA degenerate primers); rep1 or rep2 (replicates), cp (coat protein).

Some of the primers used were designed in the laboratory according to recommended standard guidelines and sent to Life Technologies-GIBCO BRL for synthesis

(Chiemsombat *et al.*, 1992; Nakhla *et al.*, 1998). Each primer had to be specific to a particular annealing region of the viral DNA genome as depicted in Figure 3.9 and 3.11.

3.2.2.2.2.3 Agarose Gel Electrophoresis

The PCR DNA product extracted above was then run in 1% agarose gel electrophoresis in TBE (Tris-borate-EDTA) buffer, 0.5% water, to determine successful amplification of the desired DNA fragment. DNA was stained with bromophenol blue using 2 µl of dye to 6 µl of PCR DNA product. A total of 8 µl was dispensed to each mini-gel well and run at 95 - 119 volts for ca. 15 - 20 min. Gels were then stained with ethidium bromide, 0.5 µl/ml, for 5 min and destained in water for 5 min (Czosnek *et al.*, 1988; Nakhla *et al*., 1998). Stained gel was viewed under Ultra Violet light and photos taken with a digital computerized camera, Pharmacia Biotech (Teare, 1996).

3.2.2.2.3 Cloning and Sequencing

Using DNA amplified in PCR experiments, cloning which is the multiplication of DNA in a plasmid vector (Lewin, 1997) was done to purify DNA prior to sequencing. Cloning was done for isolates IG1, K1 and RL5, which had reacted positively to geminivirus degenerate primers. Primers used in cloning included PAL1v 1978 and PAR1c 715, PARAv 494 and PARAc 1048, and specific primers PTYCRv 21 and PTYCRc 287, which are also used to test for genus *Begomovirus* in the western hemisphere. TOPO®-TA cloning kit protocol was used (Promega, 1996) for cloning. Plasmid pCR 2.1 TOPO® vector was used in a ligation mixture of 0.5 – 2 µl of fresh PCR product, 4 µl of disodium water and 1 µl of the vector. One Shot® bacterial cells of *Escherichia coli* were used as cloning agents in 2 µl of 0.5M β-mercaptoethanol and 250 µl of SOC medium (Invitrogen, 1998) or 1 x YT medium as substitute (Nakhla, personal communication, 1998). The One Shot® bacterial cells of *E. coli* were transformed by exposure to ice cold (4 ºC) temperatures for 30 min; heat shock for 30 seconds at 42 ºC, and ice cooling for 2 min. The mixture was incubated at 37 ºC on a horizontal rotor for 30 min.

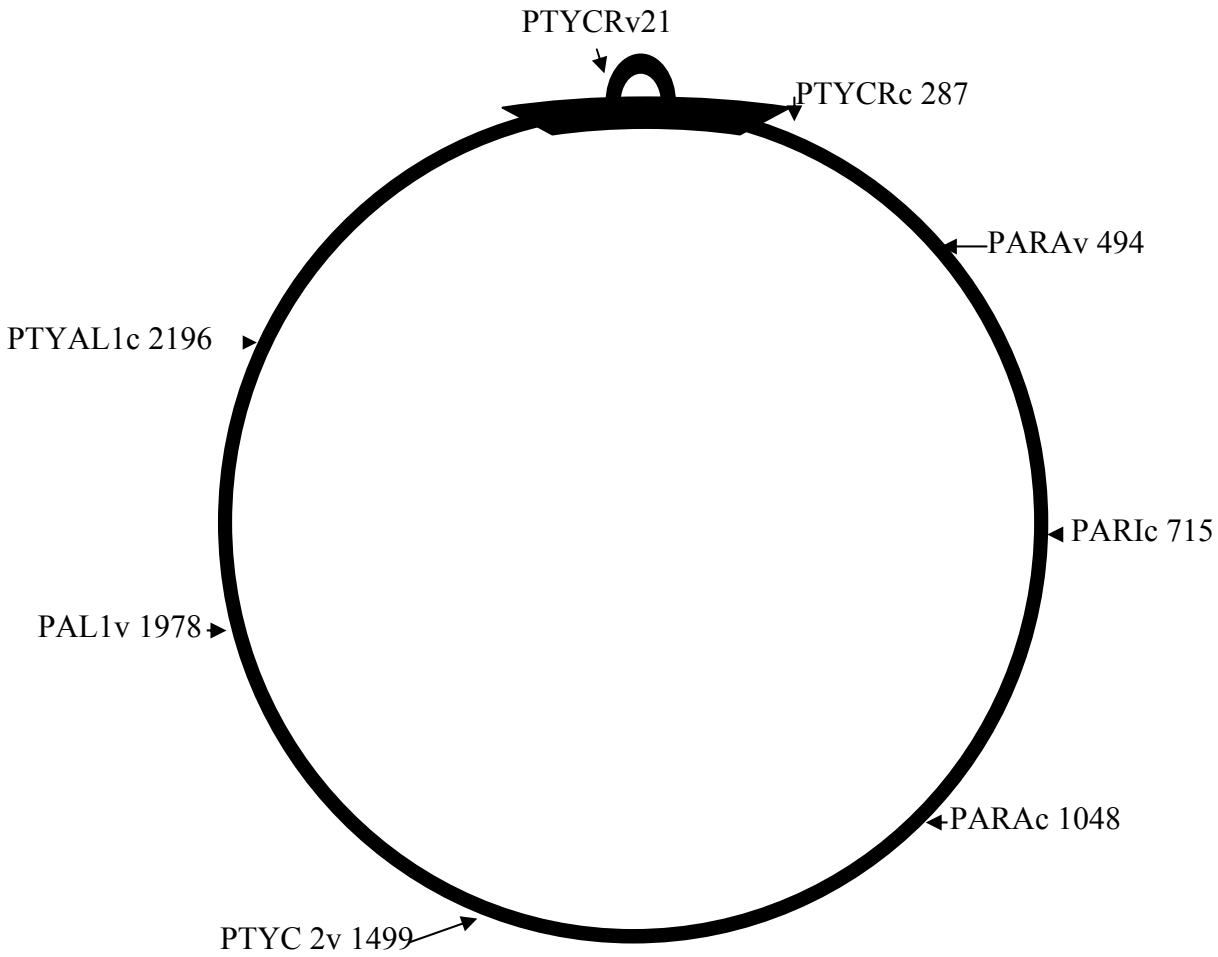


Figure 3.7: Different primers used in molecular analysis experiments and approximate points where they start annealling on a single stranded DNA (ssDNA) genome of TYLCV, and in reverse or forward direction depending on the DNA section to be amplified.

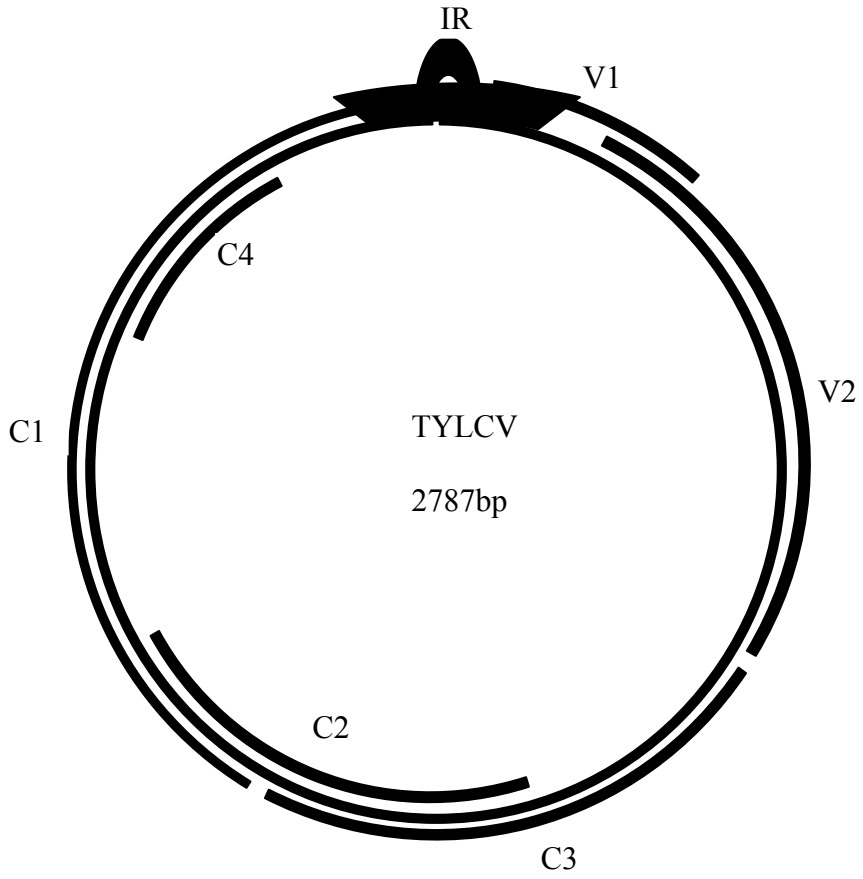


Figure 3.8: A sketch drawing of the genome organization for *Tomato yellow leaf curl virus,* a begomovirus from Israel (Navot *et al*., 1991). Open reading frames (ORFs) are V = viral sense polarity, for the coat protein gene (CP); C = complementary sense polarity, for the replication gene (Rep) whereas 1, 2, 3 or 4 refer to open reading frame numbers. IR is the intergenic region and nucleotide number one is at the beginning of this region. It is along these regions that primers used and presented in Table 3.3 above, anneal (Figure 3.7).

Clones were spread on LB medium with Ampicillin and X-Gal coating, in plastic petridishes. Inoculated plates were incubated overnight at 37 ºC (Maniatis *et al*., 1992; Nakhla *et al*., 1993). Clones could grow on media with antibiotics because of pCR 2.1 TOPO® vector plasmid (Promega, 1996), which has a gene called "ampRgene or bla" resistant to ampicillin and kanamycin (Lewin, 1997).

However, by inserting the PCR linear DNA fragment in the plasmid, the gene responsible for breaking down X-Gal (lacZgene) was destroyed. This gene is responsible for synthesis of β-galactosidase, which digests X-Gal leading to formation of blue colonies. Therefore, only white colonies were picked and individually subcultured in new tubes with 2xYT liquid media, plus ampicillin at 37 ºC, overnight[3].

Using the standard minipreps protocol used by Maxwell Laboratory (University of Wisconsin-Madison, 1998) DNA was extracted from cloned cultures and used for restriction enzyme digestion. Restriction enzyme digestion products were run on a gel, 1% agarose, and in TBE buffer. Where two bands similar to those formed with tomato leaf sample isolate RL5 DNA (ToLCV-UG) were formed again, the desired band was cut carefully from the stained agarose gel under UV light and put in a clean eppendorf tube. Agarose gel extraction protocol (QIAEX II, 1997) was used to purify DNA from the agar.

Subsequently, restriction enzyme digestion was done in order to determine whether transformation had been successful and the desired DNA fragment had been cloned. Enzyme *Eco*R1 in Multi-Core buffer was used in a reaction mixture of 6.5 µl of disodium water, 2 µl of miniprep DNA, 1 µl of multi-core buffer, and 0.5 µl of the enzyme (Srivastava *et al.*, 1995). Enzyme *Spe* 1 was also used for IG1 DNA (ToLCV-UG). The reaction mixture was incubated in a water bath for 4 hours overnight at 37 ºC. The digestion product was run on 1% agarose gel, in TBE buffer, at 95-119 Volts. Specific sites for enzyme digestion are as shown in Figure 3.9.

[3] This cloning procedure was in use by Maxwell Laboratory, University of Wisconsin-Madison. Similar literature can also be found in Lewin (1997) publication on GENES.

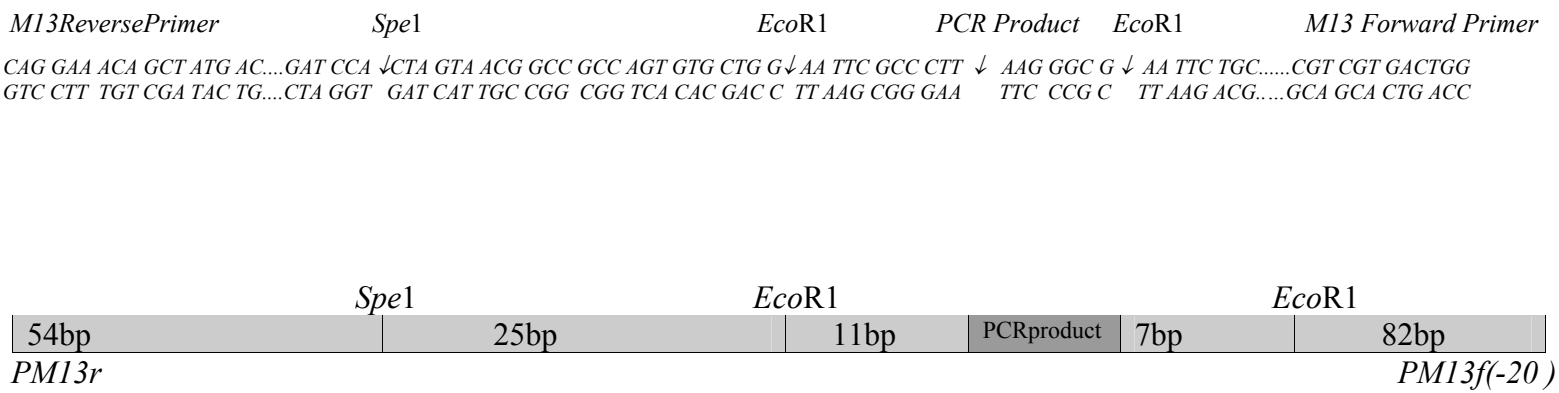


Figure 3.9: Part of a linear map of pCR2.1 TOPO vector and sites of action for restriction enzymes used, *Spe* 1 and *Eco*R 1(Invitrogen, 1998), and related restriction sites map (Clark and Russell, 1997)

Clones that had clear separation of plasmid DNA and insert DNA bands on the gel were subcultured in LB medium with ampicillin and incubated overnight at 37 ºC (Nakhla *et al.*, 1993). Mature cultures were harvested according to the Wizard Plus SV® Minipreps Spin Column protocol. Using a Promega Kit, pure plasmid DNA with the insert DNA fragment was extracted for sequencing. Of the purified DNA, 20 µl were sent to the University of Wisconsin, Biotechnology Centre for sequencing. For the DNA to qualify for sequencing, its concentration had to be between 200–300 ng/µl (Rojas *et al*., 1993). Therefore, purified DNA concentration readings for tomato leaf sample isolate IG1 DNA (ToLCV - UG) upper region and tomato sample isolate RL5 DNA (TYLCV-UG) common region were measured using a fluorimeter (Starna, UK). To sequence IG1 DNA (ToLCV-UG) upper viral sense/coat protein and RL5 (TYLCV-UG) common region, primers M13 forward and M13 reverse (Biotechnology Centre primers) were used. Primer TLCV-UG rep1f, which was sequenced during this study, was used to sequence IG1 DNA (ToLCV-UG) common region.

3.2.3 Host Range of Two Major Viruses

To further understand identified viruses TYLCV and ChiVMV, alternative host weeds and crops found in the tomato agro-ecosystem, were studied.

3.2.3.1 Tomato Yellow Leaf Curl Viruses Host Range

Weed and crop samples bearing leaf curl and/or mottling symptoms were collected from the tomato field environment (Table 3.4 and 3.5). Their sap was used in TAS- ELISA tests (Credi *et al.,* 1989) or blotted onto nylon membrane for DNA hybridisation (Czosnek *et al.*, 1988) in the laboratory. A ToLCV-UG probe, labelled using Gene-A labelling kit from Promega, was used under high stringency hybridisation conditions (65º C). Membranes were exposed to Kodak Bio Max MS film with Bio Max Transcreen LE for 24 hours, whereupon the film was developed to show results.

For TAS-ELISA (Macintosh *et al.,* 1992; Roberts *et al.*, 1984), a monoclonal antibody developed by Dr. Steven Walker (Germany) against TYLCV-SR and named TYLCV - 4F 11 C5-C3, together with ACMV IgG, was used to test samples. Nunc® microtiter plates were used.

Table 3.4: Cultivated crops and weeds bearing leaf curl and mosaic symptoms in and around tomato fields of Kabale and Kasese districts, and tested for TYLCV in the laboratory

Plant	Source	Description
Solanum melongena	Kabale Highland	Leaf mottling symptoms
Cucurbita maxima	Kasese Irrigation Scheme	Severe mosaic symptoms
Nicotiana tabacum	Kasese Irrigation Scheme	Leaf curl, mottling and stunting
Desmodium spp.	Kasese Irrigation Scheme	Small leaves with interveinal yellowing
Commelina benghalensis	Kasese Irrigation Scheme	Small leaves with mottling
Conyza floribunda	Kabale Highland	Leaf curl and crinkling
Crassocephalum spp.	Kabale Highland	Crinkled leaves with mottling
Euphorbia heterophylla	Kasese Irrigation scheme	Small curled leaves with whiteflies
Ageratum conyzoides	Kabale Highland	Mild mottling

Table 3.5: Weeds tested for tomato leaf curl virus (ToLCV-UG) begomovirus: these weeds had yellow leaf curl symptoms, and were therefore collected from Iganga and Mbarara districts

Weed	Source	Description
Euphorbia heterophylla	Iganga	Small leaves with whiteflies
Physalis floridana	Iganga	Small leaves, curl, yellow mottle
Desmodium spp.	Mbarara	Small leaves with yellowing

Samples were squashed in eppendorf tubes using a surface-sterilised prototype of Kontes pestles developed from plastic comb teeth with a broad base and small tip. Each sample was replicated twice on the microtiter plate and each test repeated twice. A sample was considered positive if both replicates turned obviously yellow in microtiter plate wells,

and the positive sample also turned yellow on the same plate, while the negative sample and blank wells remained clear.

3.2.3.2 Chilli Veinal Mottle Virus Host Range

Weed samples and other crops showing veinal mottle/mosaic symptoms were collected from within and around tomato fields. These samples belonged to species *Ageratum conyzoides*, *Aspilia africana, Bidens pilosa*, *Colocasia esculenta*, *Commelina benghalensis*, *Crassocephalum* spp., *Galinsoga parviflora*, *Lantana camara*, *Manihot esculenta*, *Ocimum basilicum*, *Phaseolus vulgaris*, *Sesbania sesban*, *Solanum nigrum*, and *Sonchus oleraceus.*

In the laboratory, samples were sliced into small pieces and wrapped in labelled filter paper over anhydrous calcium chloride. After 9 months, dried weed samples were tested for the presence of ChiVMV using DAS-ELISA (Clarks and Adams, 1977). A sample was considered positive if both replicates turned obviously yellow in microtiter plate wells, and the positive sample also turned yellow on the same plate, while the negative sample and blank wells remained clear.

3.3 Results

In this section, results of serological tests conducted to ascertain mottling and mosaic symptoms observed in tomato fields and causal viruses, which are also found in countries neighbouring Uganda, are presented. Results of molecular tests conducted for those viruses that could not be tested using serological techniques are also presented. Lastly, alternative host plants found in the tomato agro-ecosystem and with susceptibility to tomato viruses are reported upon.

3.3.1 Incidence of Virus-like Symptoms in the Field

Symptoms observed in the field were mosaic, mottling, and curl. Others were not easy to describe as they showed an overlap of different symptoms, and so were referred to as "miscellaneous". Leaf curl virus disease-like symptoms were most prevalent with a mean incidence of 42%, especially in Iganga (66%) and Kasese (66%) districts, followed by leaf mosaic with 26% and then mottling symptoms with 13%. Mosaic was most common in Mpigi (45%), whereas mottling occurred most frequently in Kasese with 36% (Table 3.6). In Kabale 87% of the symptoms had miscellaneous symptoms characterized by general leaf yellowing, with only 6% leaf curl and 7% mosaic symptom-bearing samples, and because of this high percentage of miscellaneous one in Kabale, we get the 19%.

Table 3.6 Percentage number of samples showing characteristic symptoms of tomato viruses as observed per district surveyed in 1997-1998

Districts/ Symptoms	***Rakai***	***Mpigi***	***Iganga***	***Mbale***	***Mbarara***	***Kasese***	***Mukono***	***Kabale***	***Mean***
Mosaic	31	45	4	43	36	0	39	7	26
Mottling	10	16	0	26	19	36	0	0	13
Curl	49	37	66	20	43	66	52	6	42
Miscellaneous	10	2	30	11	2	6	7	87	19

3.3.2 Confirmation of Symptoms and Identification of Viruses

3.3.2.1 Confirmation of Virus Symptoms Through DAS-ELISA

In order to confirm the fact that symptoms observed on tomato were indeed due to viruses, serological tests were conducted. DAS-ELISA results indicated that there was no symptom specific to particular groups of viruses. Samples that tested positive for ToMV, AMV, PVMV, and TSWV either had mosaic, mottling, crinkling or leaf curl symptoms. Viruses occurred singularly or in mixtures whereby two or more viruses infected one

sample (Table 3.7a). On the other hand, viruses ToMV, AMV, and PVMV were consistently associated with green and yellow mosaic or mottling, while CMV was associated with shoestring, crinkling, mottling, stunting and small leaves with marginal yellowing symptoms.

Identified viruses occurred in tomato singly, or in mixtures of two, three and more (Tables 3.8). Furthermore, during this study, twenty combinations of mixed virus infections were found in 58 tomato samples. The most frequent mixtures consisted of ToMV and PVMV, followed by ToMV and PVY (Table 3.8). Amongst districts surveyed, Kasese, Mbarara, and Mpigi had the highest number of viruses in mixed infection, i.e. showed the highest number of samples identified to have more than one virus in them. Although Mbale had low frequency of mixed infections, symptoms were very severe (score 5). During August to November of 1997 and March to July of 1998, incidence of yellow mosaic/mottling was 100% in tomato fields visited in Mpigi and Mbale Districts. By using ChiVMV antisera, it was found that 50% of collected samples contained ChiVMV alone (Figure 3.4b-A), while one sample tested positive to both PVMV and ChiVMV (Figure 3.4b-B,C and 3.10B). A weed sample of *Ocimum basilicum* bearing yellow mosaic/mottling symptoms, similar to those normally induced by ChiVMV, tested positive to PVMV and was negative to ChiVMV (Figure 3.10A).

3.3.2.1.1 Ribonucleic Acid (RNA) Viruses Identified Through DAS-ELISA

Depending on symptomatology and response to the previous test, some samples were tested more than once. In the first experiment, tomato leaf samples, which tested positive through DAS-ELISA were infected by ToMV, AMV, PVMV, CMV, TSWV, PVY, and PVX (Tables 3.7b). Of a total of 776 samples tested, 518 were positive to at least one tomato virus. Thus, 42% was positive to ToMV, 21% to AMV, 17% to PVMV, 8% to CMV, 5% to TSWV, 4% to PVY and 3% to PVX. In the second experiment, 174 samples which were showing mainly leaf curl and mottling symptoms, 48% were infected with 23% PVMV, 16% ToMV, 2% TSWV, 4% AMV, 1% PVY and 2% CMV. None of these samples tested positive to PVX. ToMV, PVMV, and AMV positive samples were

categorized as having moderate incidence (21-49% extent of infection). Of the same lot, 52% of samples with distinct virus like symptoms tested negative to antisera of seven tomato viruses known to infect tomato in neighbouring countries and ChiVMV. It is very likely that some of the infections observed on these samples are due to other viruses whose antisera were not included in the test or else to viruses that would require other tests, for example geminiviruses.

Considering identified viruses incidence and distribution, AMV was the most widely distributed virus, whereas ToMV had the highest incidence, even though it was of moderate nature (in the range of 21-49%). Kasese district had the highest virus incidence (Table 3.7 b). All seven viruses, i.e. ToMV, AMV, PVMV, CMV, TSWV, PVY and PVX, were found individually or in various combinations infecting 72 samples collected from Kasese, which had also the highest frequency of viruses occurring in mixed infections (Table 3.8). Mbarara with 66 positive samples was the second district to have virus-infected tomatoes, with an average incidence of 19% (Table 3.7b). Iganga and Mbale had lowest incidence of tomato viruses, while tomato in Rakai was mostly infected with ToMV (Table 3.7b).

Finally, in the third experiment of 52 tomato samples out of a total of 64 yellow mosaic/mottling samples collected from Mpigi district tested positive for ChiVMV. A total of 40 samples tested positive to both ChiVMV and PVMV, out of the 52 positive samples. It was interesting to note that out of 52 samples tested, 12 samples were positive to ChiVMV only. This indicates that PVMV and ChiVMV are different viruses, although sometimes found together in one infected sample (Figure 3.4b-B,C; and Table 3.8). One other notable sample was collected from Mbale District and tested using DAS-ELISA. It produced a weak reaction to TSWV, but later tested strongly positive to ChiVMV. AMV was also identified from samples with yellow mosaic/mottling symptoms similar to those induced by ChiVMV.

Figure 3.10 A) *Ocimum basilicum* sample showing yellow mottling ChiVM symptoms; (B) tomato showing stunting symptoms and was positive to PVMV and ChiVMV.

In spite of positive results reported above, a total of 102 infected tomato samples tested negatively in DAS-ELISA. However, all negative samples bore yellow mottling and leaf curl symptoms. It could not be established as to why these samples were negative. As such, section 12.2.1.2 deals with identification of other causal agents of yellow mottling and leaf curl symptoms on tomatoes.

Table 3.7 a) Grouping of samples according to nature of infection (single, mixed or negative) in 1997 and 1998.

District	No.of samples		Mixed Infection		Single infection		Negatives	
Years	1997	1998	1997	1998	1997	1998	1997	1998
Rakai	100	-[1]	4	-	45	-	51	-
Mbale	100	25	7	3	12	9	81	12
Iganga	50	4	3	2	15	2	32	0
Mpigi	50	33	11	0	13	24	26	11
Kasese	50	-	13	-	22	-	15	-
Mbarara	50	3	21	3	20	0	9	0
Mukono	50	-	0	-	6	-	44	-
Kabale	-	16	-	12	-	9	-	0

[1](-) sign stands for absence of samples collected from that district

Table 3.7 b) Incidence of tomato viruses detected in the seven districts surveyed in Uganda, in 1997 and 1998, expressed as percentage number of samples testing positive to a particular virus (% extent of infection).

Viruses	AMV		CMV		PVMV		PVX		PVY		ToMV		TSWV	
Years/19	97	98	97	98	97	98	97	98	97	98	97	98	97	98
Rakai	2	-[1]	2	-	2	-	0	-	0	-	47	-	0	-
Mbale	9	0	1	0	1	11	0	0	0	0	8	3	6	1
Iganga	22	0	14	4	0	0	0	0	0	1	0	0	0	0
Mpigi	20	0	26	0	20	19	0	0	0	1	40	2	4	2
Kasese	44	-	2	-	18	-	6	-	6	-	52	-	16	-
Mbarara	44	-	0	5	42	3	2	0	14	-	66	3	0	0
Mukono	4	-	0	-	6	-	0	-	14	-	2	-	0	-
Kabale	-	5	-	0	-	7	-	0	0	0	-	16	-	0

[1] (-) sign stands for absence of samples collected from that district

Table 3.8 Frequency of twenty mixed virus infections found in tomato samples collected from surveyed districts of Iganga (IG), Kasese (KA), Mbarara (MR), Mpigi (MP), Mbale (MB), Mukono (MU), and Rakai (RA)

Virus combinations	Districts							Frequency[1]
	IG	KA	MR	MP	MB	MU	RA	
AMV, CMV	0	0	0	0	2	0	0	2
AMV, CMV, PVMV	0	0	0	1	0	0	0	1
AMV, CMV, PVMV, ToMV, TSWV	0	0	0	2	0	0	0	2
AMV, CMV, PVMV, ToMV	0	0	0	3	0	0	0	3
AMV, CMV, PVX	0	0	0	0	1	0	0	1
AMV, CMV, ToMV	0	0	0	1	0	0	0	1
AMV, CMV, ToMV, TSWV	0	1	0	0	0	0	0	1
AMV, PVMV, PVX, ToMV, TSWV	0	2	0	0	0	0	0	2
AMV, PVMV, PVX, TSWV	0	1	0	0	0	0	0	1
AMV, PVMV, ToMV	0	0	0	1	0	0	0	1
AMV, PVMV, TSWV	0	1	0	0	0	0	0	1
AMV, ToMV	1	1	0	1	0	0	0	3
AMV, TSWV	0	1	0	0	0	0	0	1
ChiVMV, PVMV	0	0	0	40	0	0	0	40
CMV, ToMV	1	0	0	2	0	0	0	3
PVMV, PVY	0	0	2	0	0	0	0	2
PVMV, PVY, ToMV	0	0	1	0	0	0	0	1
PVMV, ToMV	2	2	13	0	0	0	4	21
PVX, ToMV	0	0	1	0	0	0	0	1
PVY, ToMV	0	3	4	0	0	0	0	7
ToMV, TSWV	0	0	0	0	0	6	0	6

[1] Total number of samples with that particular mixture

3.3.2.1.1.1 Alternative Hosts of some identified RNA Viruses

Most suspected weed samples tested negative to ToMV, AMV, PVMV, CMV, TSWV, PVY and PVX antisera. However, a number of weed samples tested positive to ChiVMV, and were considered as its alternative hosts as reported below.

ChiVMV Host Range

DAS-ELISA results indicated that among tested weed samples, i.e. *Ageratum conyzoides*, *Bidens pilosa*, *Colocasia esculenta*, *Commelina benghalensis*, *Sesbania sesbani*, *Galinsoga parviflora*, *Lantana camara*, *Ocimum basilicum*, and *Sonchus oleraceus,* were infected by ChiVMV. Healthy looking samples, which were collected from tomato fields and from the surrounding ecosystem, tested negative.

3.3.2.1.2 Dioxyribonucleic Acid Viruses Identified on Tomato

The 102 tomato samples that did not test positive to viruses of genera *Potyvirus, Tospovirus*, *Cucumovirus, Alfamovirus* and *Potato virus* X (Table 3.2 above), were tested and characterized using DNA Hybridisation, Polymerase Chain Reaction (PCR) and Gel electrophoresis molecular techniques under the supervision of Dr. M. Nakhla and Prof. D. Maxwell, at the University of Wisconsin-Madison.

3.3.2.1.2.1 Samples Infected by Members of Genus *Begomovirus*

Of the 18 diseased tomato samples showing yellow leaf curl and yellow mottling, which were squash-blotted onto nylon membranes and tested against the specific TYLCV probe, only the TYLCV positive control tested positively (Figure 3.11D and the regend table 3.10). Where genus *Begomovirus* radioactive BGMV DNA general probe was used, closely related results were obtained with both overnight and 2 days X-ray film exposure (Figure 3.11). Squash-blotted samples, i.e. IG10/22/7, IG1/3/9, IG7/22/7, ISO 30 plus TYLCV and BGMV positive controls, tested positively to the general probe at low stringency (42 ºC) as indicated in Figure 3.11A.

Furthermore, in another experiment the specific TYLCV probe was used to check for TYLCV among samples bearing leaf curl and mosaic virus like symptoms, and 13% of samples tested were strongly (dark black) positive, whereas 7% were mildly (light black) positive. Overnight X-ray film exposure results were slightly weaker than those obtained after 2 days exposure at -80 ºC as indicated in Figures 3.11E and 3.11F. For another set of squash-blotted membranes, strong positives (darker black spots) were obtained by mixing the general probe with the specific probe (Figure 3.11B and C). Using samples that had tested positively in previous DNA hybridisation reactions, overnight exposure at –80 ºC gave darker spots results than 6 hours exposure under similar conditions. Samples IG1, RL5, K3, RL1, Rl2, ISOPOT, MB2, MB7, MB8, and MB9 tested positively to the mixed probe, and were therefore confirmed to be members of genus *Begomovirus* (Figures 3.11B and 3.11C). Refer to Table 3.9which, shows samples that tested positive

Samples IG1 and K1 (Table 3.2) tested positively to TYLCV-Is with degenerate primers PAL1v 1978 and PAR1c 715 (Rojas *et al*., 1993; Nakhla *et al*., 1993; Wyatt and Brown, 1996), whereas seven more samples, i.e. IG2, IG3, RL1, RL2, K1, K3, and MB8 were positive with degenerate primers PARAv 494 and PARAc 1048 in PCR (Figure 3.12 A,B, C, Dand G), and were therefore identified to be geminiviruses.

In another experiment where specific primers to TYLCV-Is were used to test for TYLCV, 5 samples were positive with primers PTYCRv 21 and PTYIRc 287 (Figure 3.12 F), while 3 samples tested positive with primers PTYCR2v 1499 and PTYAL1c 2196 (Figure 3.12 G). No isolate was positive with primers PTYC2c 1814 and PTYV2v 466, as indicated in Figure 3.10 H. Samples ISOPOT and MB8, which clearly tested positive with primers PTYC2v 1499 and PTYAL1c 2196, did not form clear expected bands in this PCR reaction with specific TYLCV primers PTYCRv 21and PTYIRc 287, even though earlier on they were positive to the same primers in PCR (Table 3.11). Of the 10 samples tested with specific primers PTLCV-UGrep2r and cp1f, only three isolates tested positive, as indicated in Figure 3.12 I. Results of both DNA Hybridisation and PCR are summarised in table 3.11. Samples that tested negative in all experiments were disregarded in this report.

Genetic Identity of Tomato Yellow Leaf Curl Samples IGI, KI and RL5

Yellow leaf curl samples IGI, KI and RL5 taken in 1998 (Table 3.2), and which consistently tested positively in DNA hybridisation and PCR experiments, were selected for further analysis. Further more, sample IG1 drew our attention because it also reacted to the general probe, while many other samples did not (Figure 3.9A). As such, cloning and sequencing was done for IG1, KI and RL5 isolates. Partial sequences of IG1 and RL5 isolates were successfully generated. The sequence for K1 had many uncertain nucleotides and was therefore disregarded.

Isolate IG1

Isolate IG1 tested positive in PCR with primers PAL1v1978 and PAR1c715 (Figure 3.12 C). It was also positive when primers PARAv 494 and PARAc 1048 were used. Purified sample IG1 DNA was cloned with a TOPO vector, and when sequenced gave a forward viral sense sequence of 482 nt in size, and a reverse coat protein sequence of 521 nt. The coat protein was part of the upper part of the circular DNA presented in Figure 3.13. From these partial sequences, primers PTLCV-UGrep2r, PTLCV-UGcp1f and PTLCV-UGcp1r were synthesized (Table 3.12).

Isolate RL5 (DNA Intergenic Region)

In PCR, isolate RL5 reacted positively to TYLCV-Is specific primers (PTYCRv 21 and PTYCRc 287) as indicated in Figure 3.12E, and was also positive to TYLCV-Is in an experiment with primers PTYC2v 1499 and PTYAL1c 2196 (Figure 3.13F and Table 3.13). Purified DNA (fluorimeter reading of 268 ng/µl) of type isolate RL5 amplified with primers PTYCRv 21 and PTYIRc 287 for the TYLCV-Is common region auto produced a 277 nt TYLCV sequence (excludes vector and primers segments as well as overlapping) (Figure 3.14). From these results, isolate RL5 was positive to TYLCV-Is, and was therefore tentatively named TYLCV-Ugandan isolate (TYLCV-UG).

Table 3.9: Reaction of squashed and dot-blotted tomato geminivirus samples to the general, specific, and mixed probes (See figure 3.11 A, C, D, and F)

Sample	Over night exposure		**2 Days Exposure**	
Code	General probe BGMV	Mixed probe BGMV + TYLCV-EG	General probe BGMV	Specific probe TYLCV-EG
RL1	0[3]	+[1]	0	++
MB7	0	+	0	+
RL2	0	++	0	++
MB9	0	+	0	+
MB2	-[2]	+	-	+
K3	0	++	0	+++[1]
RL5	-	+	-	++
MB6	0	-	0	+
IG2	-	-	-	+
IG1	++	+	++	++
IG7	+	0	+	0
IG10	+	0	+	0
MB8	0	+	0	++
ISO30	+++	0	+++	0
ISOPOT	0	+	0	++
TYLCV	+++	+++	+++	+++
BGMV	+++	-	+++	-
Healthy	-	-	-	-

[1] (+, …, +++) intensity of positive reaction; [2](-) negative reaction; [3] (0) means that the sample was not tested with that particular probe.

Table 3.10: Legends for DNA hybridisation membranes shown in Figure 3.11: (a) Refers to squash-blotted membranes A and D; (b) refers to dot-blotted membranes E and F; (c) refers to dot-blotted membranes B and C.

a) Legend for squash-blotted membranes A (general probe) and D (specific probe)

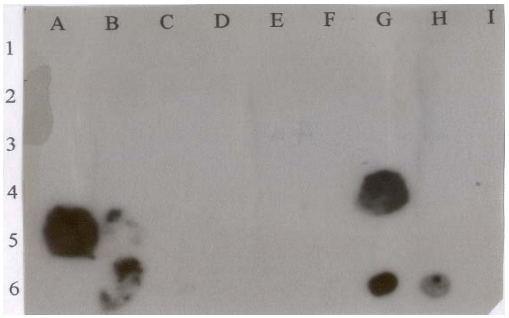
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
1	MB1 28/5/98	MB2 28/5/98	MB3 28/5/98	MB4 28/5/98	ISO 2	ISO 3	ISO 4	ISO 5	ISO 6
2	ISO 6	ISO 7	ISO 8	ISO 9	ISO 10	ISO 11	ISO 12	ISO 13	ISO 14
3	ISO 15	ISO 16	ISO 17	ISO 18	ISO 19	ISO 20	ISO 21	ISO 22	ISO 23
4	ISO 24	ISO 25	ISO 26	ISO 27	ISO 28	ISO 29	ISO 30	ISO 31	Healthy
5	IG 10/ 22/7	IG 7/ 22/7	MB 4/ 22/7	MB 20/ 22/7	MB 2/ 22/7	MB 5/ 22/7	MB 3/ 22/7	MB 10/ 22/7	MB 21/ 22/7
6	RL 5	IG 1 3/9/98	IG 2 3/9/98	IG 2 3/9/98	IG 2 3/9/98	K2 3/9/98	TYLCV	BGMV	-ve

b) Legend for dot-blotted membranes E and F (specific probe)

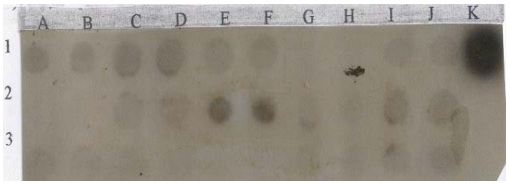
	A	B	C	D	E	F
1	IG1	IG2	RL5	K2	K3	Healthy
2	CVMV	K1	RL1	RL2	ISOPOT	Diseased
3	MB8	CVMVmx	MB6	MB9	MB7	Healthy

c) Legend for dot-blotted membranes B and C (mixed probe)

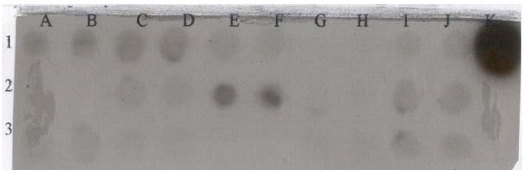
	A	B	C	D	E	F	G	H	I	J	K
1	RL1	RL1	RL2	RL2	RL5	RL5	K2	K2	IGI	IG1	TYLCV
2	KN	KN	MB9	MB9	K3	K3	IG2	IG2	MB8	MB8	BGMV
3	MB7	MB7	MB2	MB2	IG3	IG3	MB6	MB6	ISOPOT	ISOPOT	Healthy



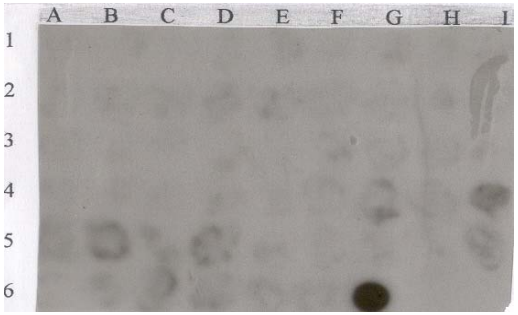
A) X-ray film with reactions of squash-blotted samples, hybridised with the general probe



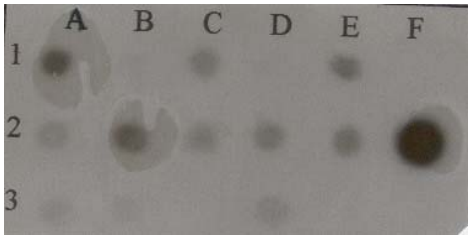
B) Reaction of dot-blotted tomato samples to the mixed probe, 6 hours exposure



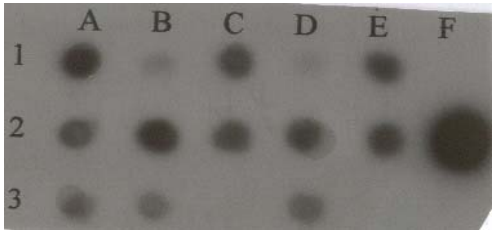
C) Reaction of dot-blotted tomato samples to the mixed probe, overnight exposure



D) Squash-blotted membranes and their reaction to the specific TYLCV probe



E) X-ray membranes showing results of membranes hybridised with a specific probe, overnight



F) X-ray membranes showing results of membranes hybridised with a specific probe, two days exposure

Figure 3.11: DNA hybridisation results For regends see table 3.10

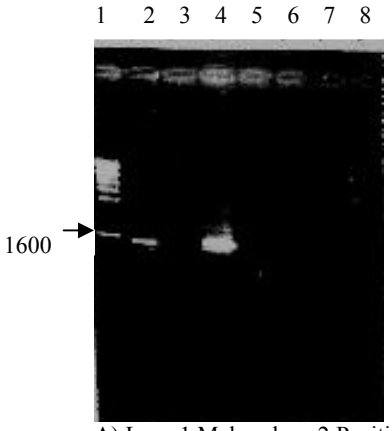
Table 3.11: Summary of DNA hybridisation and PCR results, also showing cloned and sequenced samples

Samples	Hybridisation		Polymerase Chain Reaction (PCR)					Cloning and Sequencing		
			Primer PAv 494 and Pac 1048	TYLCV-EG (TYLCV-Is)			TLCV-UG Specific primers	TYLCV	CP TYLCV	TLVC-UG
				PTYCRv21 and PTYCRc287	PTYCRv 1499 and PTYCRc 2196	PTYCRv 466 and PTYCRc 1814	PTLCV-UGrep2r and PTLC-UGcp1f			
RL1	-	+[1]	-[2]	-	+	0	-	0	0	0
RL2	-	+	-	++	+++	0	-	0	0	0
RL5	++	+	+	++	+++	-	-	+	0	+
K2	+	-	0	0[3]	0	0	0	0	0	0
IG1	+++	+	+++[1]	0	++[1]	0	++	-	0	+
KN	-	-	0	0	0	0	0	0	0	0
MB9	+	+	++	0	+	0	-	0	0	0
K3	++	++	-	+++	+++	-	+	0	0	0
IG2	-	-	0	0	0	0	0	0	0	0
MB8	-	+	++	+++	+++	0	-	0	0	0
MB7	-	+	0	0	0	0	0	0	0	0
MB2	-	-	0	0	0	0	0	0	0	0
IG3	+	-	0	0	0	0	0	0	0	0
MB6	-	-	0	0	0	0	0	0	0	0
ISOPOT	++	+	+++	+++	+++	0	-	0	0	0
CVMV	+	0	-	0	+++	0	-	0	0	0
CVMVmxd	+	0	++	0	+	0	0	0	0	0

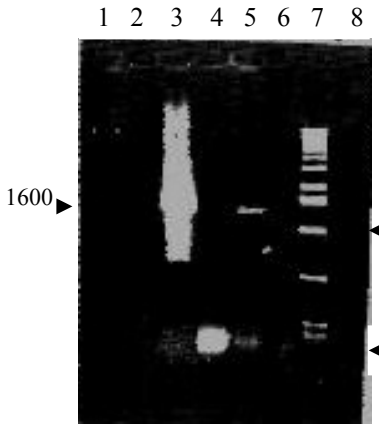
([1]+,…, +++) = intensity of positive reaction to geminiviruses' test; (-)[2] = negative reaction to geminiviruses' test; [3] (0) stand for samples that were not included in that particular experiment.

Figure 3.12 Reaction of tomato virus samples to degenerate primers PAL1 v 1978 and PAR1c 715: (**A** and B) 1% agarose in TBE buffer, at 97 volts; (C) reaction of tomato samples to coat protein degenerate primers PAR Av 494 and PAR Ac 1048: 1% agarose in TBE buffer, at 97volts; (D and E) agarose gel (1%) electrophoresis, reaction of PCR product DNA for selected samples where primers PTYCRv 21 and PTY IR c287 were used; (F and G) agarose gel (1%) electrophoresis photo showing reaction of PCR product DNA for selected samples using primers PTYC2v 1499 and PTYAL1c 2196; (H) agarose gel (1%) electrophoresis results photo showing reaction of PCR product DNA using primers PTYCrc 1814 and PTYV2v466; (I) agarose gel (1%) electrophoresis results, showing reaction of PCR product DNA for selected samples with primers PTLCVUGrep2r and cp1f

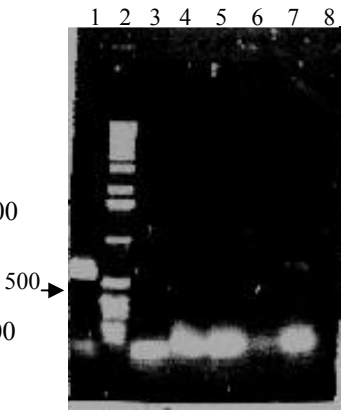
NB: Photos were labelled according to the 1kb Marker Ladder from Biotechnology Research Laboratory (BRL), Wisconsin-Madison, USA



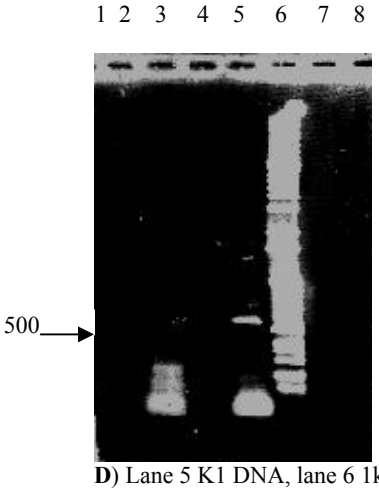
A) Lane 1 Maker, lane 2 Positive sample, lane 3 IG 2, lane 4 IG 1, lane 5 RL 5, lane 6 IG 3, lane 7 K 2, 8 RL 1; (x100 IGI DNA and other samples)



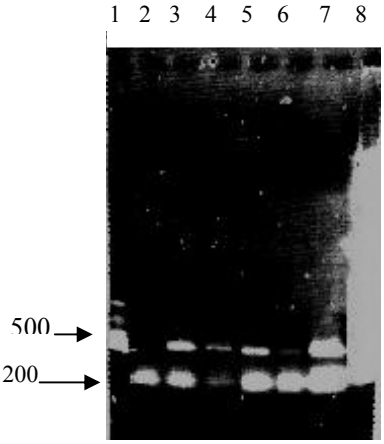
B) Lane 3 Pep GMV (+ve), lane 4 Healthy sample, lane 5 K1, lane 6 IG1, lane 7 1kb Marker, lane 8 RL1; (x1 Dellaporta K1 DNA)



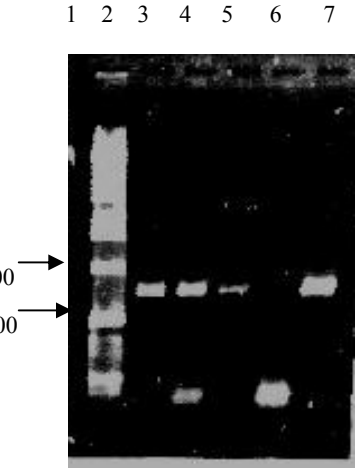
C) Lane 1 IG 1, lane 2 1kb Maker, lane 3 RL 2, lane 4 K3, lane 5 RL5, lane 6 ChiVMV, lane 7 MB8, lane 8 RL1; (Dellaporta IG1 DNA)



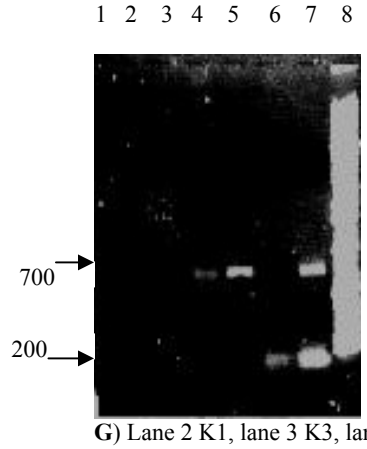
D) Lane 5 K1 DNA, lane 6 1kb Marker; (x 1 Dellaporta K1 DNA)



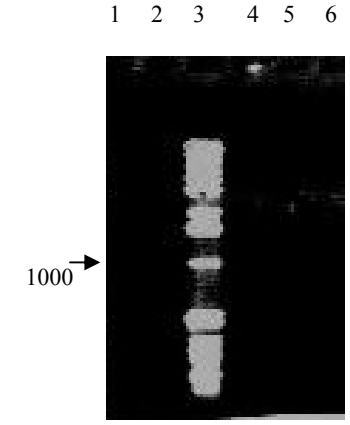
E) Lane 1 Positive, lane 2 RL1, lane 3 ISOPOT, lane 4 RL2,lane 5 MB8, lane 6 RL5, lane 7 K3



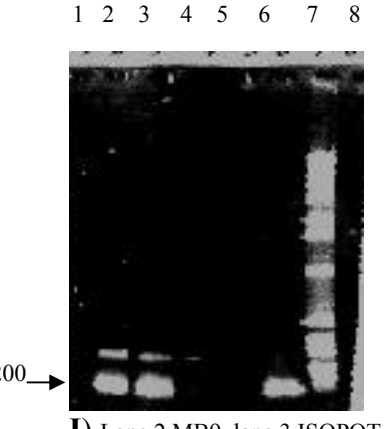
F) Lane 2 1kb Maker, lane 3 RL2, lane 4 ChiVMV, lane 5 RL5, lane 6 ChiVMV Mxd, lane 7 MB8



G) Lane 2 K1, lane 3 K3, lane 4 IG1, lane 5 MB9, lane 6 RL1, lane 7 ISOPOT, lane 8 1kb Marker



H) Lane 3 1kb Marker, lane 4ISOPOT, lane 5MB8, lane 6 K3



I) Lane 2 MB9, lane 3 ISOPOT, lane 4 ChiVMV, lane 5 MB8, lane 6 RL1, lane 7 1 kb Mark

Figure 3. 12

Table 3.12: ToLCV-UG specific primers prepared during this study

Primer Name	Sequence	Hybridisation point
PTLCV-UGrep2r	GAGAATGTCATGAGTTCCGCTGCG	2078
PTLCV-UGrep1f	GGGGATACCAGGTCGAAGAATCGG	2437
PTLCV-UGcp1f	GTATTACATAGGGTTGGCAAGAGG	641

```
-------------------------------------------ACGACTA-------TCAC 11
-------------------------------------------ACGACTA-------TCAC 11
TCA---GACACCCAACATTCAAGTTCCTCTGGAACTTGATCAAATGAAGAAGAAAGAAAA 124
GGAGAAACATA-TCCTTCAACGGAGGTGTAAAAATCTTATCTAAATTACA-TTTAAATTA 182
TGATACTGAAAAATAAAATCCTTAGGGAGTTTCTCCCTAATAATAGCCAAAGCGGCTTCA 242
GCGGAACCTGCGTTTAATGCCTCGGCGGCTGCGTCGTTAGCATTCTGGCAGCCTCCTCTA 302
GCACTTCGTCCGTCGACCTGAAATTCTCCC-ATTCGAGGGTGTCTCCATCCTTGTCGATG 361
TAGGACTTGACGTCGGAGCTGGATTTAACTCCCTGAATGTTTGGATGGAAATGTGCTGAC 421
CTGGTTGGGGATACCAGGTCGAAGAATCGGTGATTCTTGCAGGTGTATTTGCCTTCGA-C 480
TG---------------------------------------------------------- 482
```

A

```
--------------------------------TGTGGGATCCACTGTTAA---ATGAGTTC 26
CCAGACTC-TGTTC------ATGGGT-TTCG---TTGCAT-----GCTTGCTATAAAATA 70
TTTGCAGGCTATTGAGTCCA---CTTACGAGCC-CAATACTTTGGGCC--ACGATTTAAT 124
TCGA-------GAT---------------------------------CTCA--TTTGTGTC 143
GTT---AGAGCCAGAGATTATGTCGAAGCGACCCGG---AGATATAATAATTTCAACGC- 196
CCGCCTCG-AAGGTTCG---TCGAAGGTTG-----AACTTCGACAGC----CCGTACACC 243
A--GCCGTGCTGCTGTCCCC----ACTGCCCCAGGCACAAGCAGACGTCGATCATGGACT 297
TACAGGCCCATGTATCGAAAGCCCAGGATGTACAGAATGTACAGAAG-CCCTGATGTTCC 356
TCGGGGT---TGTGAAGGCCC--------ATGTAAGGTT---CAGTCGTACGAGCAGAGA 402
GATGATGTTAAGCATACTGGTGTTGTCCGTTGTGTTAGTGATGTTACTTCGTGGGTCGGG 462
TA-TTACACATAGGGTTGGCAAGAGGTTCTGTGTGAAGTCCATTTACATTATAGGGAAAA 521
```

B

Figure 3.13: Partial DNA sequence of the Ugandan begomovirus, tentatively named ToLCV-UG, which is from sample IGI. This sample was originally collected from Iganga district. The tomato had severe leaf curl symptoms. A) Forward sequence 482 bp segment of the C1, replication gene; B) viral sense polarity sequence, 521 bp segment of the coat protein gene (V1, V2), after removing primers and working on overap.

NB: Note that primers PAL 1v 1978 and PAR 1c715 were used to amplify the viral sense polarity DNA segment

```
-----------------------------------ATCGGTGTCCCTCAA----AGCT 19
CTATGGCAATCGGTGTATCGGTGTCTTACTTATACCTGGACACCTAATGGCTATTTG-GT 78
AATTTCATAAATGTTCATTGCAATTCAAAATTCAAAATTCAAAAATCAAATCATTAAAGC 138
GGGCATCCGTATAATATTACCGGATGGCCGCGCCTTTTCCTTTTATGTGGTCCCCACGAG 198
GGTTACACAGACGTCACTGTCAACCAATCAAATTGCATCCTCAAACGTTAGATAAGTGTG 258
CATTTGTCTTTATATACTT---------------------------------------- 277
```

Figure 3.14: Partial DNA sequence of TYLCV-UG (RL5 DNA), 277 bp of the intergenic region (IR) amplified using specific primers PTYIRv21 and PTYIRc715, after primer and overap segments are worked on.

3.3.2.1.3 Host Range of Tomato Yellow Leaf Curl Viruses

ToLCV-UG was subsequently detected in *Euphorbia heterophylla*, *Physalis floridana* and *Desmodium* spp. (Table 3.13a). At least six weed species associated with the tomato agro-ecosystem were confirmed to be infected by TYLCV-UG, that is, *Desmodium* spp., *Commelina benghalensis*, *Conyza floribunda*, *Crassocephalum* spp., *Euphorbia heterophylla* and *Ageratum conyzoides*, as shown in Table 3.13b. Healthy samples of these plants tested negative.

Table 3.13: Samples of alternative hosts that tested positive to (a) ToLCV-UG; and (b) TYLCV-UG (strain of TYLCV-Is, TYLCV-IR and TYLCV-SR)

(a) ToLCV-UG

Sample Code/ Collection Date	Alternative Host	Test result (DNA hybridisation) ToLCV-UG
IG14/24.03.98	*Euphorbia heterophylla*	+++[1]
IG17/24.03.98	*Physalis floridana*	+++
MBl/28.04.98	*Desmodium* spp.	+
IG1 positive	*Lycopersicon esculentum*	+++
HTS	negative weed sample	-[2]

[1]Positive to test virus; [2] negative to test virus

(b) TYLCV-UG (strain of TYLCV-Is, TYLCV-IR and TYLCV-SR)

Sample Code/ Collection Date	Alternative Hosts	Test Result (TAS-ELISA) Mab. TYLCV-SR (TYLCV-4-4F-11 C5-C3)
2/Kabale/22.9.99	*Solanum melongena*	+
23/Kabale/23.9.9	*Cucurbita maxima*	+
22/Kasese/23.9.9	*Nicotiana tabacum*	+
3/Kasese/23.9.9	*Desmodium spp.*	+
8/Kasese/23.9.9	*Commelina benghalensis*	++
4/Kabale/22.9.99	*Conyza floribunda*	+
17/Kabale/22.9.9	*Crassocephalum spp.*	+
20/Kasese/23.9.9	*Euphorbia heterophylla*	+
14/Kabale/22.9.9	*Ageratum conyzoides*	++
RL5 positive	*Lycopersicon esculentum*	+++

3.4 Discussion

In surveyed districts of Mbarara, Mpigi and Kasese, which had mosaic symptoms and many tomato virus species in mixtures, also had high incidence of mottling and leaf curl symptoms (Table 3.6). Therefore, the occurrence of virus mixtures (Table 3.8) may have a synergistic relationship with virus symptom development and incidence. Gallitelli (2000) reported similar mixed infections in tomato by CMV, AMV and TSWV for Apulia, Italy. In our case, mottling symptoms on tomato leaves could not be associated with any of the latter three viruses, because all of them contributed to this appearance. Consequently, one cannot rely fully on symptoms to unequivocally identify tomato viruses, even though some viruses are named according to symptoms (Martyn, 1968; Bock, 1982; Green *et al.*, 1991).

Furthermore, both host tomato plants and alternative hosts (Annex 3) in the farming system contribute to vector population growth and dynamics, as also observed in Sudan by Moustafa (1991). During our study, it was observed that tomato farmers in Kasese district irrigated their fields (Annex 3). Because of irrigation, tomato farmers in Kasese

are able to grow tomatoes throughout the year. Such a situation enhances conditions for vector population build up and virus spread to new fields. Rotation with non-susceptible alternative crops, especially during periods of poor returns from the tomato crop and in the absence of susceptible weeds, breaks the epidemiological cycle (Wisler *et al*., 1997). Therefore, rotation is recommended in such situations.

This study led to identification of ten tomato viruses by employing serological and molecular tests to identify causal agents of mosaic, mottling, veinal mottle and leaf curl symptoms on Ugandan tomato, which is grown in farming conditions such as those mentioned in the first two paragraphs above. These ten viruses included potyviruses ToMV, PVMV, ChiVMV and PVY, potexvirus PVX, alfalfamovirus AMV, cucumovirus CMV, tospovirus TSWV, begomovirus TYLCV-UG (TYLCV-Is strain), plus a begomovirus tentatively named ToLCV-UG, and were not very different from what is reported to occur elsewhere in East Africa. Indeed, TSWV was identified in East Africa as early as 1969 (CMI, 1969); ToMV was reported to be seed-borne and occurs worldwide (Brunt *et al*., 1990); Nono-Womdim *et al*. (1996) reported ToMV to be widely present in East Africa; finally PVMV was reported to infect pepper in Kenya (Brunt *et al*., 1990), but Nono-Womdim *et al*. (1996) did not detect it in Tanzania. However, Nono-Womdim *et al*. (1993) and Ladipo *et al*. (1979) reported PVMV to infect pepper in West Africa, while Reccah, (1986) reported PVMV to be (*Myzus persicae*) aphid-transmitted in a non-persistent manner. AMV was reported to occur in Europe and America where mottling, interveinal yellowing, yellow-whitish mosaic and fruit distortion were observed (Brunt *et al*., 1990; Jones *et al*., 1991). Where AMV individually infected tomato in Uganda, symptoms observed were also typically mottling, whitish yellow mosaic and fruit distortion.

In our experiments virus incidence was ToMV (31%), AMV (1%), PVMV (13%), CMV (6%), TSWV (1%), and PVY (0.3%), respectively. This was the first research record on tomato viruses in Uganda. It was also the first identification of AMV on tomato in Uganda. PVX was also reported on tomato in Uganda for the first time. The limitation of

PVX's host range to members of family *Solanaceae* (Green, 1991) may be responsible for its observed low incidence in Uganda. In contrast, the high incidence of ToMV in Uganda, could have been due to its being seed-borne (Brunt *et al.*, 1990), and due to the fact that some farmers, especially in Rakai district, extract their own seed for subsequent cropping cycles (Annex 3). The survey showed that in Iganga district, where farmers only used certified seed, ToMV did not occur. ToMV was observed to cause severe mosaic symptoms, which were earlier on reported by Defrancq (1989) and Hansen (1990). The wide distribution and high incidence of ToMV were considered a big threat to tomato growers, and chances of spreading the disease farther are very high in a situation where farmers collect and preserve their own seeds. Broadbent (1965, 1976) reported that ToMV was easily spread with seed, running water and wind. Consequently, it is recommended to identify ToMV strains existing in Uganda in order to compare them with strains present elsewhere in the region.

Furthermore, *Tomato spotted wilt virus* was detected in only three of the eight districts surveyed despite high thrips populations at the time (Annex 3). It could have been possible that some of these thrips were feeding on tomatoes, and were not carrying any TSWV inoculum. Literature revealed that TSWV was identified in East Africa as early as 1969 (CMI, 1969). Therefore, the need to study the vector *Frankliniella* spp., and to identify TSWV strains, using procedures reported by Verkleij and Peters (1983), Gonslaves *et al.* (1989), and Wijkamp *et al.* (1995), is recommended for future research activities.

Likewise, Brunt *et al.* (1990) reported *Pepper veinal mottle virus* in Kenya. Even though Kenya is a neighbour of Tanzania, Nono-Womdim *et al.* (1996) did not detect it on tomato in Tanzania. However, during our study, PVMV was also identified in Ugandan tomato. During the survey, PVMV incidence was only 8%. Most times PVMV was occurring in combination with ToMV. This was the most frequent mixed tomato virus infection, as shown in table 3.8. PVMV symptoms were observed to be mild everytime it was singly infecting a tomato plant, but showed severe green mosaic or mottling

whenever it occurred in mixed infection with ToMV or ChiVMV. As such, PVMV alone can be considered not to be a very dangerous virus on tomato.

During our study, eight viruses were tested by serology (Table 3.7). As reported earlier, some samples did not react positively, even though they bore clear viral symptoms. Those viruses were especially from Mbale and Mpigi districts. Samples of this kind tested positive to ChiVMV as shown in table 3.7. From our literature review it is evident that there is little information on this virus (Brunt *et al*., 1990). Furthermore and to the best of our knowledge, ChiVMV has not been reported from tomato in Africa (CMI, 1970; Brunt *et al*., 1990, Hansen, 1990; Green *et al*., 1991; Mwaule, 1995). Green *et al.* (1991) described ChiVMV as a *Potyvirus* (750 nm). Its alternative hosts were reported to be solanaceous plants such as green pepper (*Capsicum annuum*), and that it is transmitted in a non-persistent manner by aphids (Brunt *et al*., 1990). ChiVMV is also known to infect chilli peppers (*Capsicum* spp.) in Malaysia, where it causes dark green mottling and small fruits, and up to 50% yield loss. Other countries where the virus is reported in pepper are Taiwan, Thailand, Korea, Philippines and Indonesia (Green *et al*, 1991). However, this is the first report of ChiVMV on tomato in Uganda probably because ChiVMV antisera had not been available before and nobody ever took trouble to test for it.

Tomato plants with a mixed infection of PVMV and ChiVMV expressed symptoms similar to white-yellow mosaic caused by AMV. Consequently, the need for future detailed characterization of ChiVMV isolates is herewith recognized. Samples from plants expressing mosaic symptoms, similar to those induced by ChiVMV, were also found to be positive to AMV. AMV symptoms can easily be confused with ChiVMV symptoms, and therefore, it is recommended to always check for both viruses in samples with such symptoms, especially when using serological techniques.

Even after successfully identifying ChiVMV, there were samples with virus disease like leaf curl and mottling symptoms, which still tested negative to any available antisera and

probes against viruses mentioned above. According to Cohen *et al*. (1966) and Wisler *et al*. (1998), there could be viruses belonging to families *Geminiviridae* and *Closteroviridae*, which cause tomato leaf curl and mottling symptoms. Czosnek and Laterrot (1997) also reported tomato yellow leaf curl viruses (*sensu lato*) to be a complex of virus species belonging to family *Geminiviridae*. Brunt *et al*. (1990) attributed tomato mosaic and mottling to families *Tombusviridae*, *Luteoviridae*, *Potyviridae*, *Bromoviridae* and *Bunyaviridae*. Pringle (1999) reported virus families with viruses that infect tomato to be *Geminiviridae*, *Bunyaviridae*, *Potyviridae*, *Bromoviridae*, *Closteroviridae*, and *Luteoviridae*. It is known from the Virology website that there are 146 virus species infecting tomato (http://image.fs.uidaho.edu/vide/descr002.htm). As such, there is high probability that unidentified viruses could belong to families reported elsewhere or may even be new viruses (Bock, 1982). Therefore, advanced molecular techniques, such as polymerase chain reaction (PCR) (Navot *et al*., 1992) were very useful for us to confirm that other tomato samples expressing leaf curl and mottling symptoms were infected by geminiviruses. Even though two samples CVMV and CVMVmxd reacted positive to TYLCV-Is in DNA Hybridisation (Table 3.11), it was not possible to test all the 800 samples, including those, which had reacted positive in serology because of limited resources. It could be possible that TYLCV exists in mixture even with other RNA viruses.

The fact that some samples bearing leaf curl and mottling symptoms were positive in PCR with general primers used to test for geminiviruses and were not positive with TYLCV-Is-specific primers, indicates that among leaf curl symptom-bearing samples, there were geminiviruses other than TYLCV-Is. Isolate IG1, which tested positive with degenerate primers (Table 3.3) only, could have been infected with a whitefly transmitted begomovirus, which causes tomato leaf curl. This causal virus was tentatively named ToLCV-UG. ToLCV-UG was confirmed a new virus by Shih *et al*. (2005). Thus, ToLCV-UG samples, which were collected and sent to the World Vegetable Centre (AVRDC, Taiwan), again proved different from any other geminiviruses, under complete sequence analysis and comparison experiments. Another of our own isolates, i.e. RL5,

tested positive to TYLCV-Is specific primers and produced a 277 nt partial sequence. It was therefore tentatively named TYLCV-UG until when a complete genome sequence is produced.

In conclusion, molecular techniques like PCR could be used in Uganda as a second step towards identifying viruses that cannot be identified using serological techniques. Furthermore, ToLCV-UG causes leaf curl, severe stunting, no fruiting and dieback of infected tomato plants, whereas TYLCV-UG causes stunting, leaf curl and/or mottling, small leaves, dieback, flower drop, whereas small fruits are formed on at least one truss per plant. Flower abscission and small fruit size have also been reported to be characteristics of tomato yellow leaf curl viruses in general (Mazyad *et al.*, 1979; Czosneck *et al.,* 1988). On the other hand, yellow leaf curl is a symptom caused by a complex of virus species (Moriones and Navas-Castillo, 2000). Therefore, the lack of any clear difference between symptoms caused by TYLCV-UG and ToLCV-UG implies that symptoms are non-characteristic and therefore are not a basis for geminivirus differentiation.

On testing for ToLCV-UG using specific primers, few samples reacted positive, whereas others only produced weak bands. Such results could be attributed to low concentrations of viral DNA in sample plants (Nono-Womdim *et al*., 1996). The amplification of small, undesired DNA bands, detected in agarose gel electrophoresis of ToLCV-UG PCR product, when ToLCV-UG specific primers were used, would be attributed to mispriming (Briddon and Markham, 1994). This indicates the need to design specific primers that can amplify large DNA fragments of ToLCV-UG. However, ToLCV-UG-specific primers, currently available with Maxwell Laboratory, University of Wisconsin-Madison, were shown to be useful for testing for ToLCV-UG.

In enzyme digestion experiments, using *Eco*R1, ToLCV-UG fragment size of 1.4 kb was amplified. Full size TYLCV DNA is known to be 2.8kb (Pringle, 1999; Kheyr-Pour *et al*., 1999). According to Clark and Russell (1997), failure of restriction enzyme *Eco*R1 to

digest ToLCV-UG and to form the same DNA bands as those formed by digested TYLCV-Is DNA, implies that both viruses differ from each other. Different TYLCV (*sensu lato*) strains have also been reported in other African countries. These include South Africa, Senegal, Tanzania, Malawi, Zambia, Zimbabwe, Nigeria, Ivory Coast, Egypt and Sudan (Yassin *et al.*, 1982; AVRDC, 1987; Czosnek *et al.*, 1990; Nakhla *et al.*, 1992; AVRDC, 1993; Nono-Womdim *et al.*, 1996). In Tanzania, another tomato leaf curl virus, i.e. TLCV-Tz or ATLCV (Chiang *et al.,* 1996) was identified in tomato.

Furthermore, studying host range of major viruses identified in Uganda was indeed important. This part of our study established that TYLCV-UG hosts were *Ageratum conyzoides, Commelina benghalensis*, *Conyza floribunda*, *Cucurbita maxima, Desmodium* spp., *Euphorbia heterophylla*, *Nicotiana tabacum,* and *Solanum melongena*. At the same time, ToLCV-UG was identified in *Desmodium* spp., *Euphorbia heterophylla,* and *Physalis floridana*. As evident from studies conducted since 1966 (Cohen and Antignus, 1994; Nakhla *et al*., 1994; Mansour, 1992; Ioannu, 1987; Nakhla *et al*., 1978; Nitzany, 1975; and Cohen *et al*., 1966), TYLCV is known to have at least 28 different alternative hosts belonging to plant genera *Datura*, *Chrysophyllum*, *Cynanchum*, *Hibiscus*, *Hyoscyamus*, *Lycopersicon*, *Malva*, *Nicotiana*, *Sesamum*, *Solanum*, and *Vitis*. Nono-Womdim *et al*. (1996) identified TYLCV-Is in *Achyranthes aspera*, *Euphorbia heterophylla*, and *Nicandra physaloides*. Therefore, our own and previous studies confirmed the occurrence of TYLCV in solanaceous plants and *Euphorbia heterophylla*. All other alternative hosts were reported for the first time. This was also the first report of ToLCV-UG in *Desmodium* spp., *Euphorbia heterophylla*, and *Physalis floridana* in Uganda.

In the case of RNA viruses, host range was considered only for ChiVMV, whose host range elsewhere seemed not to be well studied. Green (1991) and Black *et al*. (1991) identified ChiVMV on chilli pepper (*Capsicum* spp.), and established that the host range of ChiVMV was limited to members of family *Solanaceae*, contrary to our findings. Indeed, ChiVMV was identified in *Ageratum conyzoides, Bidens pilosa*, *Colocasia*

esculenta, *Commelina benghalensis*, *Galinsoga parviflora*, *Lantana camara, Ocimum basilicum, Sesbania* spp., and *Sonchus* spp.. It was the first time ChiVMV was detected in weeds found in the tomato agro-ecosystem in Uganda.

All the above weeds are commonly found in the tomato agro-ecosystem as observed during the survey (Annex 3). Weed hosts of TYLCV-UG and ToLCV-UG are not very different from weed hosts of other tomato geminiviruses elsewhere (Nono-Womdim *et al*., 1996; Cohen *et al*., 1994; Nakhla *et al*., 1994; Mansour, 1992; Ioannu, 1987; Nakhla *et al*; 1978; Nitzany, 1975; Cohen *et al*., 1966). Existence of alternative hosts may explain early infection of tomato plants by tomato yellow leaf curl viruses (*sensu lato*) and *Chili veinal mottle virus*. Therefore, this information serves as justification for regular weed management in tomato fields, as an Integrated Pest Management (IPM) option for the sustainable control of TYLCV-UG, ToLCV-UG and ChiVMV.

Considering the damage observed, the mode of transmission and the wide occurrence in alternative hosts, tomato leaf curl (*sensu lato*) is taken to be an economically important tomato virus disease, and will therefore be the focus of subsequent chapters 4 and 5. These chapters deal with Ugandan leaf curl viruses' (TYLCV-UG and ToLCV-UG) relationship with other geminiviruses reported elsewhere in the world, and with their whitefly vector.

CHAPTER 4

HOMOLOGY BETWEEN TOMATO LEAF CURL VIRUSES IDENTIFIED IN UGANDA AND OTHER TOMATO LEAF CURL VIRUS ISOLATES IDENTIFIED ELSEWHERE IN THE WORLD

In this chapter, the objective is to study the relationship between TYLCV-UG and ToLCV-UG, which are found on tomato in Uganda, with other tomato leaf curl viruses found elsewhere in the world

4 CHAPTER 4

Homology Between Tomato Leaf Curl Viruses Identified in Uganda and Other Tomato Leaf Curl Virus Isolates Identified Elsewhere in The World

4.1 Introduction

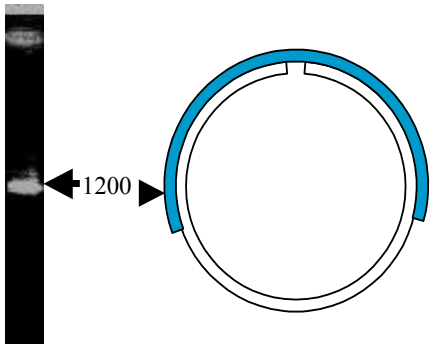
Variations and similarities have been reported among tomato yellow leaf curl viruses (TYLCVs) and tomato leaf curl viruses (ToLCVs) in both New and Old World (Padidam *et al*., 1997). Padidam *et al*. (1995) found that phylogenetic trees obtained from the alignment of nucleotide sequences of 36 geminiviruses using the Phylogenetic Analysis Using Parsimony (PAUP) programme and the Un Weighted Pair Group Method with Arithmetic mean (UPGMA) distance matrix/neighbourhood-joining method of the MegAlign programme, had New and Old World viruses clustering separately. On the same phylogenetic trees, TYLCVs and ToLCVs clustered together within the old world geminiviruses cluster, but formed separate subclasses. Brown (1997) argued that these similarities and variations could still be detected using partial sequences of the intergenic region and the coat protein gene. Gorsane *et al.* (2003) used the same approach to differentiate members of the tomato yellow leaf curl virus complex in Tunisia. Following Brown's argument, TYLCVs and ToLCVs were identified to be begomoviruses and members of sub-group III of the Old World begomoviruses, but were genetically different.

Begomoviruses have spread within the Old World and have also been identified on tomato and capsicums in the New World. Thus, in the New World, TYLCV was identified in Cuba (Accotto *et al*., 2001), in southern Florida (Ying and Davis, 2000), and in Mexico (Ascencio-Ibanez *et al*., 1999). In the Old World, TYLCV (*sensu lato*) strains, which are members of genus *Begomovirus*, have been reported in South Africa, Senegal, Tanzania, Malawi, Zambia, Zimbabwe, Nigeria, Ivory Coast, Egypt and Sudan (Yassin *et*

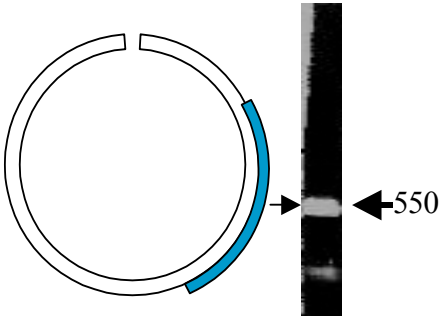
al., 1982; AVRDC, 1987; Czosnek *et al.*, 1990; Nakhla *et al.*, 1993; AVRDC, 1993; Nono-Womdim *et al.*, 1996). In Tanzania, Chiang *et al.* (1996) identified another tomato leaf curl virus (ATLCV-TZ). Later, a ToLCV-related virus, *Tobacco leaf curl virus* (TbLCZbwV), was isolated from tobacco in Zimbabwe by Paximadis and Rey (2001), while Pieterson *et al*. (2000) identified a new begomovirus, in South Africa, *Tomato cury stunt virus*, which formed cluster with TYLCV-Is.

It is with a clear identification of the causal organism that appropriate virus management options can be drawn (Bock 1982). If this can be achieved with the intergenic region and coat protein gene sequences comparisons with sequences in the (EMBL/NCBI) genbank (Brown, 1997), this chapter deals with comparison of begomoviruses found in Uganda with those already identified elsewhere and that have their DNA sequence in the geminiviruses genbank (Fauquet *et al*., 2003; Moriones and Navas-Castillo, 2000). If this is successfully done, Ugandan TYLCV and ToLCV should be understood in terms of their homology with other species of genus *Begomovirus.* Furthermore, the use of the intergenic region and coat protein gene sequences would hence be proven to be useful and cheaper, as a first step approach, than the full sequence comparison approach (Fauquet *et al*., 2003).

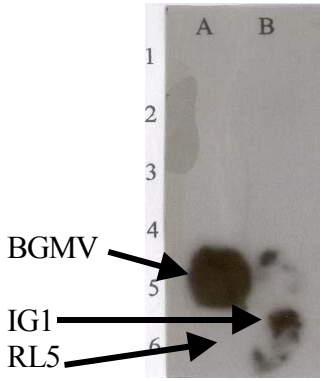
From our tomato yellow leaf curl viruses identification molecular experiments in Chapter 3 above, many samples tested positive. However, samples IG1 and RL5 were notable because of the different way they reacted to experimental probes. In DNA hybridization, IG1 tested positive to the Bean golden mosaic virus degenerate probe, whereas sample RL5 was negative in this experiment (Figure 4.1C). In another experiment where specific TYLCV-Is probes were used, both IG1 and RL5 tested positive (Figure 4.1F). Similar results were generated in PCR, where IG1 reacted positve to primers pairs AL1v1978: ARc715 and ARAv494: 1048, while RL5 was negative (Figure 4.1A/B). Nevertheless, RL5 was positive in a PCR experiment with specific primers C2v 1499: AL1c2196 and CRv21: IRc287 (Figure 4.1D/E). Therefore, these two samples were selected for further characterization experiments reported about in this Chapter 4.



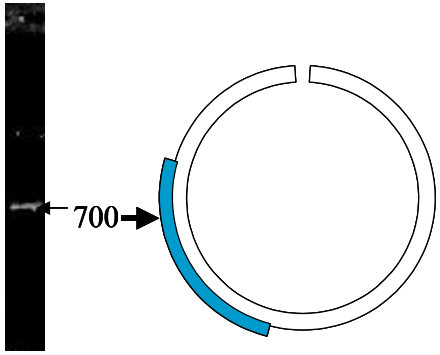
(A) IG1 (ToLCV-UG)
PAL1v 1978 and PARc 715



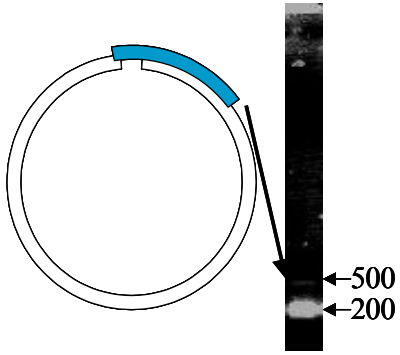
(B) IGI (ToLCV-UG)
PARAv 494 and PARAc 1048



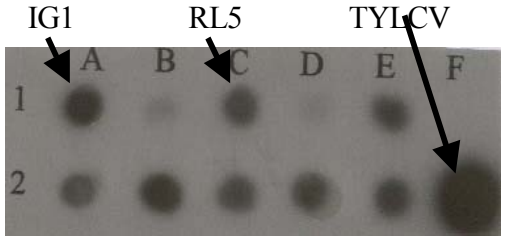
(C) General BGMV probe



(D) RL5 (TYLCV-UG)
PTY C2v 1499 and PTY AL1c 2196



(E) RL5 (TYLCV-UG)
PTY CRv 21 and PTY IRc 287



(F) Specific TYLCV probe

Figure 4.1: IG1 and RL5 varied reaction to same probes. They could be different and require more research.

4.2 Materials and Methods

4.2.1 Multiple-alignment of a 550 bp Segment of ToLCV-UG Sequence, and 360 bp IR Segment of TYLCV with Other Members of Genus Begomovirus

Using BLAST and EMBL (http://www.ebi.ac.uk/Clustalw/) Clustal W (1.82), IG1 (ToLCV-UG forward-replication gene and reverse- coat protein gene sequences), and RL5 (TYLCV-UG IR sequence) were multiple-aligned and pairwise comparison done with equal size segments of Rep. Gene, CP gene and IR of sequences from the National Centre for Biotechnology Information (NCBI) Genbank and the European Molecular Biology Laboratory (EMBL, UK) (Table 4.1). Nucleotide sequence identities between viruses used in multiple-alignment were calculated from pairwise percentage distance values (Clustal W version 1.82), and phylogenetic trees generated using full optimal alignment and bootstrap neighbour-joining method with the Clustal X software (http://www.ebi.ac.uk/Clustal x/). One thousand bootstrap replications were performed to place confidence estimates on the groups contained in trees, which were rooted using sequence information of ChaMV-Ng, Acces.No. CMV AJ 3191 for ToLCV-UG, and OyVMV-PK, Acces.No. AYO 36010.1 (Zhou *et al*., 1998) for TYLCV-UG. Phylogenetic trees were viewed using Treeview (Page, 1998).

4.3 Results

4.3.1 Genome Organization

Analysis of both TYLCV-UG partial sequence and ToLCV-UG begomovirus sequence indicated that their genome is formed of three ORFs in addition to the intergenic region (IR) as shown in figures 3.6, and 3.7. The IR is a common region for bipartite geminiviruses (Lockhart, 1990; Nakhla *et al*., 1993). The three open reading frames are viral-sense polarity coat protein gene (V1, V2) and complementary-sense polarity,

replication gene (C1). These are just part of the six ORFs, namely V1, V2 (for the coat protein) and C1, C2, C3, C4 (for the replication gene), which were revealed in a complete TYLCV-Is genome with 2787 b (Nakhla *et al*., 1993), see also Figure 3.6 above.

4.3.2 Relationship Between Sequences of ToLCV-UG and TYLCV (IR) with Other Sequences of Begomoviruses

In this section, results of pairwise comparison for ToLCV-UG, replication gene and coat protein and TYLCV-UG intergenic region (IR) homology with other begomoviruses are presented. Results indicated identity percentages less than 90% for ToLCV-UG.

4.3.2.1 Multiple Alignment, Pairwise Comparison Matrices and Identity Percentages of ToLCV-UG and TYLCV-UG Cloned Sequences with Other Viruses

From alignment matrices results, it is evident that ToLCV-UG replication gene was most identical with the replication gene of ATLCV-TZ by 89%. Other closely related geminiviruses were TbLCZbwV (88%) and TbLCZbwV-mild strain (86%). In contrast, ToLCV-UG replication gene had only 10% identity with replication gene of ToLCV-Au, which implies that the two viruses differ genetically. All virus sequences aligned with ToLCV-UG (482bp) sequence of the replication gene were identical for the nucleotides in the range of 80-205nts and differed for the segment between 10-40 nts (Annex 6.1b).

ToLCV-UG 521 bp segment composed of the IR, as well as V1 and V2 coat protein genes, was more identical to EACMV-MW with 85% < 90% identity than to any other multiple-aligned virus. ToLCV-UG was more identical to TYLCV-Is/PT, TYLCV-JR, and TLCV-SD than to ATLCV-TZ. In this case, the percentage relationship with other geminiviruses of the same DNA segment was less than 90% (Table 4.3). ToLCV-UG coat protein gene and other multiple-aligned virus sequences had consensus for nucleotides in section 300-555 nts, and differed for nucleotides in section 41-145 nts (Annex 6.1a).

Table 4.1 List of sequences used in phylogenetic analysis. The table shows their names, abbreviations, open reading frames (ORFs), and genbank accession numbers. Virus nomenclature is according to Padidam (1997) and Fauquet *et al*. (2000)

Name	**Abbreviation/References**	**Sequence ORFs/genes**	**Access. No**
African tomato leaf curl virus (Tanzanian isolate)	ATLCV-TZ (Chiang *et al*.,2001)	AV1,AV2,AC1	ATU73498
Tobacco leaf curl virus (Zimbabwe isolate)	TbLCZWV (Paximadis *et al*., 2001)	Complete genome	AF 368275
Tobacco leaf curl virus (Zimbabwe mild isolate)	TbLCZWV (Paximidis *et al*., 2001)	V1,C4 genes	AF368274
Chayote mosaic virus	ChaMV-Ng (Thottappilly *et al*.)	DNA-Acomplete genome	CMV AJ 3191
Soy bean crinkle leaf virus	SCLV(Samretwanich *et al*.,2001)	Complete genome	ABO 50781.1
Tomato curly stunt virus (S.African isolate)	TCSV-JR (Ooi *et al*., 1997)	C1 and C4 genes	AF 261885.2
Ageratum enation virus (Nepal isolate)	AEV-NP (Briddon *et al*., 2002	Complete genome	AEN437618
Okra leaf curl virus (Egypt isolate)	OkLCV-EG (Idris *et al*., 2002)	SegmentA complete genome	AYO 36010.1
Tomato leaf curl virus (Malaysian isolate)	ToLCV-MY (Shih *et al*.,1998)	SegmentA sequence	AF 327436
Tomato leaf curl virus (Philippines isolate)	ToLCV-PH	DNA-A C1, V1, V2	AF 136222
East African cassava mosaic virus (Malawi)	EACMV-MW (Pita *et al*., 2001)	DNA-A V1 gene	AJ006461
Okra yellow vein mosaic virus (Pakistan isolate)	OyVMV-PK (Zhou *et al*., 1998)	DNA-A	AJ002451
Tomato yellow leaf curl virus (Portugese isolate)	TYLCV-Is (Navas-Castillo *et al*., 2000)	Complete genome	AF 105975
Tomato yellow leaf curl virus (Lebanese isolate)	TYLCV-LB (Abou-Jawdah *et al*., 1999)	IR	AF160875
Tomato yellow leaf curl virus (Japan-Shizouka isolate)	TYLCV-JR (Kato *et al*., 1998)	DNA complete genome	ABO 14346.1
Cotton leaf curl virus (Sudan isolate)	CLCUV-SD (Idris *et al*., 2000)	Complete DNA-A	AF 260241
Tomato yellow leaf curl virus (Egypt isolate)	TYLCV-EG (Nakhla *et al*.,1993)	IR	L 12219
Tomato yellow leaf curl virus (Jamaican isolate)	TYLCV-JM (Wernecke *et al*., 1997)	C1,1R, V1,V2	U88889
Tomato yellow leaf curl virus (Cuban isolate)	TYLCV-Cu (Accotto *et al*.,2001)	C1,V1,V2	U65089
Tomato yellow leaf curl virus (Mexican isolate)	TYLCV-Mx (Ascerweio *et al*., 1999)	C1, V1, V2	***
Tomato yellow leaf curl virus (US-Florida isolate)	TYLCV-US (Ying and Davis, 2000)	C1, C4, V2, V1	***
Tomato leaf curl virus (Gezira isolate)	TYLCV (Idris *et al*.)	V2, V1, C3, C2, C1, C4	AY044138.1
Tomato leaf curl-India	ToLCV-IN	-[2]	L12738-9/Z48182
Tomato leaf curl virus-Taiwan	ToLCV-TW	-	U88692
Tomato leaf curl virus-Australia	ToLCV-AU	-	S53251
Tobacco leaf curl virus-China	TbLCV-Ch	-	AF240675

[1] stars (***) accession number not available, [2] (-) open reading frames not given

For the TYLCV-UG 277 nucleotides sequence segment of the intergenic region (IR), alignment results showed that TYLCV was almost 100% identical with TYLCV-EG, TYLCV-Cu, TYLCV-JM, TYLCV-Mx, TYLCV-US and TYLCV-LB (Table 4.4). TYLCV-UG (IR) had almost 100% consensus with other IR sequences TYLCV-EG, TYLCV-Cu, TYLCV-JM, TYLCV-Mx, TYLCV-US and TYLCV-LB. However, there were differences between TYLCV-UG and TYLCV-LB, at the CTAATG of the IR sequence section (Annex 7).

4.3.2.2 Phylogenetic Analyses

In the phylogenetic tree, ToLCV-UG (521 bp) segment of IR and V1, V2 open reading frames, which encode for the coat protein, formed one cluster with TYLCV-OM, TYLCV-IR, TYLCV-Is and TLCV-SD, with bootstrap values of 66% to 100%. Within the same cluster, ToLCV-UG formed a sub-cluster with EACMV-MW at a high bootstrap value of 100%. In contrast, ToLCV-UG coat protein gene did not cluster either with ATLCV-TZ, TbLCZbwV, TbLCZbwV-mild, OkLCV-EG, CLCUV-SD, ToLCV-MY, TbLCV-Ch nor AEV-NP with bootstrap differences of 100% – 41% (Figure 4.3).

For the ToLCV-UG (482 bp) segment of C1 replication gene, unlike the coat protein gene, a cluster was formed with TbLCZbwV, ToLCZwbV-mild strain, TCSV-SA and ATLCV-TZ with a bootstrap value of 78%. A sub-cluster was formed between ToLCV-UG replication gene and ATLCV-TZ with a branch bootstrap value of 72% (Figure 4.2). Consequently, ToLCV-UG replication gene was considered identical with ATLCV-TZ replication gene. ToLCV-UG did not cluster with ToLCV-Ph, ToLCV-MY, ToLCV-TW and ToLCV-Au, and could therefore be considered a different tomato leaf curl begomovirus. ToLCV-UG formed clusters with EACMV-MW and ATLCV-TZ, and could be a mutant of the two viruses.

Furthermore, phylogenetic analysis revealed a strong relationship between TYLCV-UG and TYLCV-EG (Figure 4.4). The two viruses formed a cluster of their own with a bootstrap value of 87%. These results confirm the high pairwise comparison identity

percentages recorded above in section 16.2.1. These tomato yellow leaf curl viruses also clustered according to New or Old World (Figure 4.4).

Table 4.2: Clustal W (1.82) multiple sequence alignment of the C1 gene of ToLCV-UGf (482bp)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
ToLCV-UGf	100	78	78	78	77	74	73	89	86	88	87	81	72	10
TbLCV-PH		100	94	92	86	81	82	79	70	77	77	72	76	10
ToLCV-MY			100	91	87	82	80	78	71	78	78	73	76	2
SCLV				100	86	81	81	79	71	79	79	72	77	9
ToLCV-PH					100	80	79	75	74	75	76	70	71	3
CLCUV-SD						100	94	77	75	78	78	73	72	3
OkLCV-EG							100	75	75	75	75	69	71	3
ATLCV-TZ								100	84	87	87	78	75	4
TbLCZWVm									100	94	93	74	67	4
TbLCZWV										100	97	81	73	4
TCSV-SA											100	80	74	3
ChaMV-Ng												100	68	3
ToLCV-TW													100	8
ToLCV-Au														100

Table 4.3: Clustal W (1.82) multiple sequence alignment of ToLCV-UGr (521bp) of coat protein genes (ORFs) IR, V1 and V2

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
ToLCV-UG	100	3	3	7	8	5	4	4	9	84	84	84	84	72	69	85
OkLCV-EG		100	98	83	76	76	74	72	75	2	2	3	3	5	12	5
CLCUV-SD			100	82	76	76	74	72	74	2	2	7	4	3	12	5
ToLCV-MY				100	79	76	78	72	82	6	6	7	3	2	12	5
TbLCZWV					100	96	87	80	80	4	4	3	2	7	12	5
TbLCZWVm						100	85	77	79	4	4	3	2	7	12	5
ATLCV-TZ							100	75	80	4	4	4	4	4	2	3
ChaMV-Ng								100	74	5	5	6	5	6	5	5
AEV-NP									100	4	4	5	5	8	19	5
TYLCV-Is										100	100	98	97	73	69	84
TYLCV-JR											100	98	97	73	69	84
TYLCV-OM												100	97	63	64	83
TLCV-SD													100	73	69	84
ToLCV-IN														100	68	73
ToLCV-TW															100	71
EACMV-MW																100

Table 4.4: TYLCV-UG, 360bp segment of the Intergenic region, Clustal W (1.82) multiple sequence alignment with TYLCV-EG, TYLCV-Cu, TYLCV-JM, TYLCV-Mx, TYLCV-US, TYLCV-LB, and OyVMV-PK as out-group

Virus	1	2	3	4	5	6	7	8
TYLCV-UG	100	99	98	98	98	98	96	66
TYLCV-EG		100	98	98	98	98	96	66
TYLCV-Cu			100	98	98	98	96	66
TYLCV-JM				100	99	98	96	66
TYLCV-Mx					100	98	96	66
TYLCV-US						100	95	66
TYLCV-LB							100	66
OYVMV-PK								100

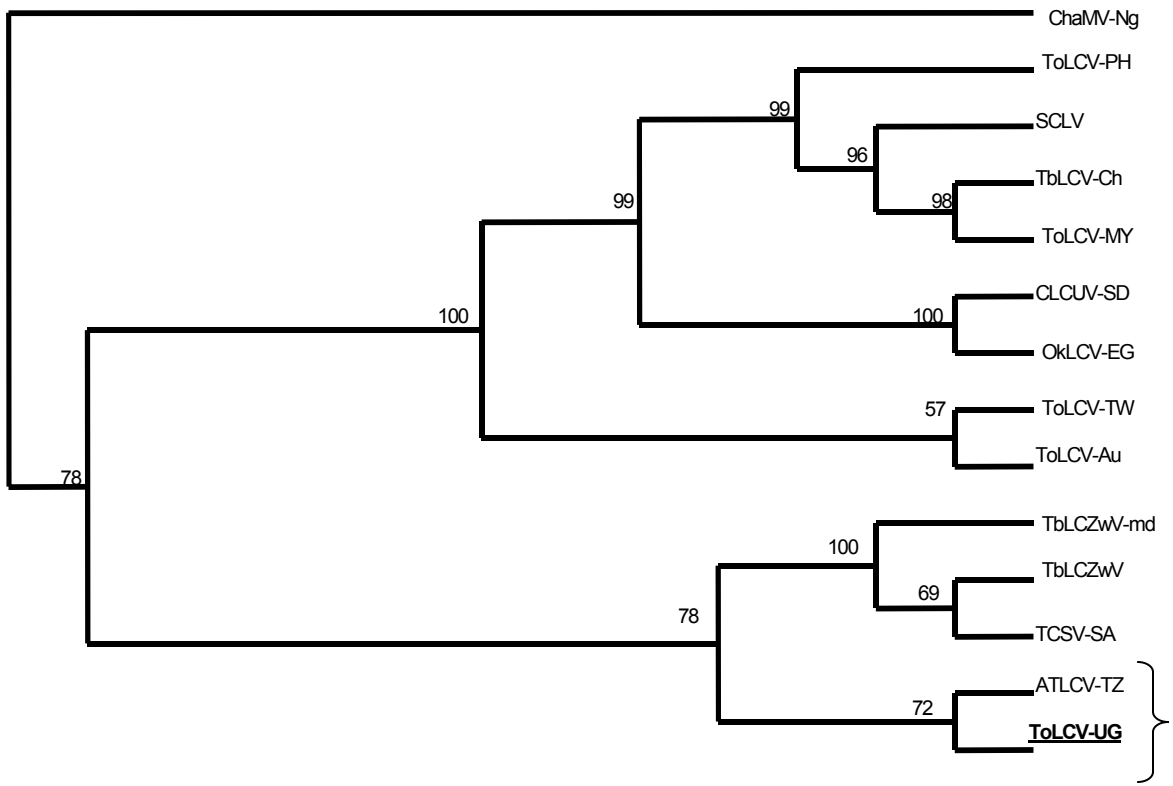


Figure 4.2: Phylogenetic tree of ToLCV-Ugf seq, C1replication gene (482bp) aligned with similar size of TbLCV-PH, ToLCV-MY, SCLV, ToLCV-PH, CLCUV-SD, OkLCV-EG, ATLCV-TZ, TbLCZWV, TbLCZWV-md, TCSV-SA, ToLCV-TW, ToLCV-Au, and ChaMV-Ng as outgroup. The tree is drawn using Clustal X and viewed with TreeView (Page, 1998). Bootstrap values are percentages of 1000. Bootstrap values were placed at various nodes, whereas nodes lacking a score are considered dubious. ToLCV-UG is underlined.

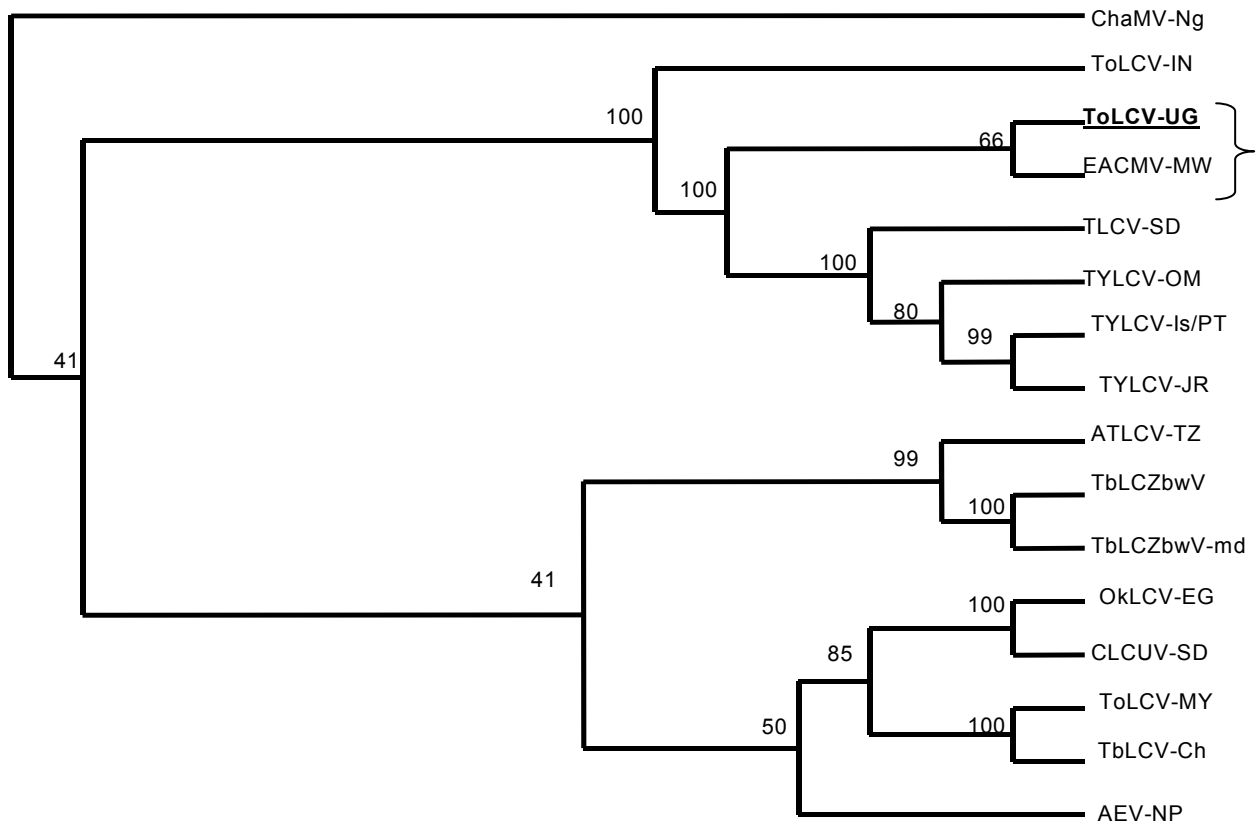


Figure 4.3 : Phylogenetic tree of ToLCV-Ugrev seq, Coat protein gene (521bp) aligned with similar size of ToLCV-MY, CLCUV-SD, ATLCV-TZ, TbLCZWV, TbLCZWV-md, ChaMV-Ng, ToLCV-TW, ToLCV-IN/Bangalore, TLCV-SD, TYLCV-Is/PT, TYLCV-JR, TYLCV-OM, and OkLCV-EG as outgroup. The tree is drawn using Clustal X and viewed with TreeView (Page, 1998). Bootstrap values are percentages of 1000. Bootstrap values were placed at various nodes, whereas nodes lacking a score are considered dubious. ToLCV-UG is underlined.

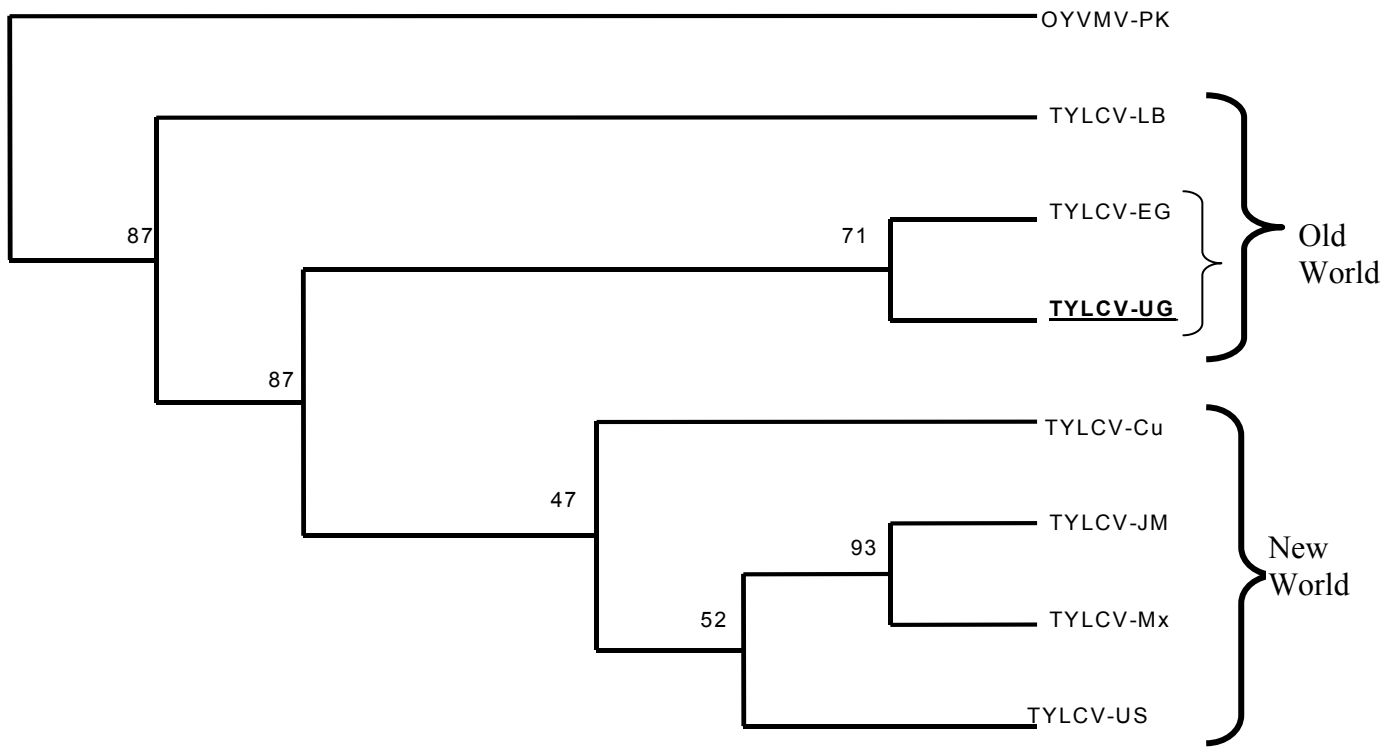


Figure 4.4: Phylogenetic tree of TYLCV-UG Intergenic region (277bp) aligned with similar size of TYLCV-EG, TYLCV-Cu, TYLCV-JM, TYLCV-Mx, TYLCV-US, TYLCV-LB and OYVMV-PK as outgroup. The tree is drawn using ClustalX and viewed with TreeView (Page, 1998). Bootstrap values are percentages of 1000. Bootstrap values were placed at various nodes, whereas nodes lacking a score are considered dubious. TYLCV-UG is underlined.

4.4 Discussion

Using intergenic region and coat protein sequences (Brown, 1997), it was possible to generate meaningful pairwise comparisons and phylogenies, as well as deeper understanding of the identities of the two geminiviruses, which we documented before in Uganda. Our study findings show that the 482 bp sequence of the C1gene of ToLCV-UG was less than 90% identical to other viruses used in the comparison and had 89% identity to ATLCV-TZ. Furthermore, ToLCV-UG (521) bp segment for the coat protein gene and parts of the intergenic region (IR) showed less than 90% identity to other viruses used in the comparison, and 85% to the EACMV-MW. Since similar geminivirus strains have identical coat protein gene, replicative gene and intergenic region (Padidam *et al*, 1995; Brown, 1997), ToLCV-UG is very closely related to ATLCV-TZ and EACMV-MW, but a different virus.

By contrast, the 277 bp sequence of the IR of TYLCV-UG was 96%-99% identical to eight genbank yellow leaf curl viruses. TYLCV-EG was 99% similar to TYLCV-UG, which is therefore taken to be a strain of the latter. Based on findings of Padidam *et al*. (1995), a break-off percentage of 90% identity, and the fact that all related geminiviruses have identical nucleotide sequences for the intergenic region, TYLCV-UG, TYLCV-EG, TYLCV-Cu, TYLCV-JM, TYLCV-Mx, TYLCV-US, and TYLCV-LB are considered similar strains of TYLCV-Is. Consequently, we can conclude that two identified begomoviruses, i.e. TYLCV-UG and ToLCV-UG, are responsible for leaf curl symptoms on tomato in Uganda. TYLCV-UG is a strain of TYLCV-Is, while ToLCV-UG can tentatively be called a begomovirus related to ATLCV-TZ, which is a yellow leaf curl virus of tomato in Tanzania, and EACMV-MW, which is a cassava mosaic virus reported from Malawi and parts of western Kenya.

The International Committee for the Taxonomy of Viruses (Mayo, 2000) considered 36 members of family *Geminiviridae* and classified them into two vector-transmission

categories, i.e. leafhopper-transmitted and whitefly-transmitted viruses. ICTV went further to divide family *Geminiviridae* into four genera. The first genus (*Mastrevirus*), with one monopartite DNA molecule, contains leafhopper-transmitted viruses of monocotyledonous plants. The type species is *Maize streak virus*. The second genus (*Curtovirus*) consists of leafhopper-transmitted viruses, which infect dicotyledonous plants. The type species is *Beet curly top virus*. The third genus (*Begomovirus)* consists of whitefly-transmitted viruses of dicotyledonous plants. These have both monopartite and bipartite members. Monopartite viruses are confined to the Old World, while bipartite viruses originally belong to the New World (Padidam *et al*., 1995). The fourth genus, *Topocuvirus*, was recently accepted by ICTV and has only one member, *Tomato pseudo-curly top virus* (Fauquet *et al*., 2003). From findings of our study, viruses ToLCV-UG and TYLCV-UG found in Uganda belong to family *Geminiviridae* and genus *Begomovirus*, and not to any of the other families and genera referred to in this document. Our recent study of similar isolates from Uganda, based on complete sequence comparisons, confirmed that the tomato leaf curl virus is new (Shih *et al*., 2005).

Since it was possible to better understand ToLCV-UG and TYLCV-UG, which cause tomato leaf curl disease in Uganda and hence reduce tomato productivity, future studies would require countrywide sample collection, and sequence pairwise comparisons as well as phylogenies of either complete DNA sequences or the intergenic region and coat protein genes, depending on available resources.

Furthermore, to develop appropriate control methods for these begomoviruses, there is a need to better understand the influence of weather conditions, tomato growth stages and tomato management practices on whitefly vector population (Anderson, 1992; Bock, 1982). These aspects of the study are dealt with in Chapter 5.

CHAPTER 5

RELATIONSHIP BETWEEN TOMATO YELLOW LEAF CURL VIRUSES AND THE WHITEFLY VECTOR

In this chapter, the objective is to establish TYLCVs (sensu lato) temporal and spatial spread, as well as their relationship with the whitefly vector in a selected tomato agro-ecosystem in Uganda

5 CHAPTER 5

Relationship Between Tomato Yellow Leaf Curl Viruses And The Whitefly Vector

5.1 Introduction

In Chapters 3 and 4, two begomoviruses, i.e. TYLCV-UG and ToLCV-UG, were identified. TYLCV-UG was found to be a strain of TYLCV-Is. ToLCV-UG was found to be a close relative of ATLCV-TZ. Since begomoviruses, including TYLCV-Is and ATLCV-TZ, are transmitted by *Bemisia tabaci* (Hemiptera: family *Aleyrodidae*) as reported by Cohen and Harpaz (1964), TYLCV-UG and ToLCV-UG could be transmitted similarly. However, according to Czosnek and Laterrot (1997), there are sometimes variations in the mode of begomoviruses transmission by the same whitefly vector. It is also reported that virus diseases themselves can sometimes disappear from a field (also referred to as random extinction) due to seasonal changes in the farming ecosystem (Garcia-Arenal *et al*., 2000). These variations were reported in Egypt (Moustafa, 1991), where variations in tomato yellow leaf curl disease incidence were observed during Spring (February-April), Summer (September-mid-October) and Autumn. Nono-Womdim *et al.* (1996) also reported on variations in whitefly population and incidence of TYLCV in Tanzania.

In general, whitefly-borne viruses of the family *Geminiviridae*, to which begomoviruses belong, were recognized as major causal organisms of tomato diseases in the early 1990s (Padidam *et al*., 1995). In East Africa, whitefly-transmitted tomato leaf curl diseases were only recently reported (Nono-Womdim *et al*., 1996; Chiang *et al*., 1996) for the first time. However, until this study, nothing was known about TYLCV's and ToLCV's epidemiology and their relationship with whitefly vectors in Uganda. Previous studies,

which were conducted in Uganda, focused on whitefly as a vector of cassava mosaic virus disease (Legg, 1996).

According to Polston and Anderson (1997), genetic changes occur in whitefly–transmitted geminiviruses. Based on this finding, and on the fact that TYLCV-UG and ToLCV-UG are related to other already known begomoviruses (Chapter 3 and 4), we considered both viruses to be transmitted by the whitefly vector. This Polston and Anderson (1997) finding further implies that there was a need to have full knowledge of pathosystems through a multi-component approach. Therefore, this chapter deals with field studies conducted with the objectives to determine: the general trend of whitefly population dynamics in relation to TYLCV disease incidence at different tomato growing stages and seasons in Buwama sub-county, Mpigi district; the effect of management practices on whitefly populations in this area; as well as the impact of management practices on TYLCV incidence and spread. In our hypotheses, it was assumed that: whitefly populations vary according to changes in weather conditions, which also influence tomato planting date, growth and development; incidence of tomato yellow leaf curl disease depends on whitefly population changes; and farmers activities, like date of planting, weeding, pesticide applications and so on, can affect whitefly populations and in turn tomato yellow leaf curl and mottling diseases incidence.

5.2 Materials and Methods

5.2.1 Site Selection

An area in Buwama sub-county, Mpigi District where 33% tomato leaf samples tested negative in DAS-ELISA (Chapter 3, table 3.7), and where later on some samples tested positive to tomato yellow leaf curl viruses in PCR, was selected for field experiments. The area has cassava (*Mannihot esculenta*), sweet potato (*Ipomea batatas*), maize (*Zea mays*) and banana (*Musa* spp.) as major crops, as well as *Physalis floridana* and *Imperata*

cylindrica as major weeds (Annex 1 and 3). It also experiences a characteristic bi-modal rainfall regime (Annex 1).

5.2.2 Experimental Design and Layout

The experiment was repeated three times between 1999 and 2000 to take care of annual dry and wet seasonal variations (Moustafa, 1991). As such, this period included two dry seasons and two rainy seasons, i.e. March to June (rainy season) and July to August 1999 (dry season), September to December 1999 (rainy season) and January to February 2000 (dry season). Each planting was done at the beginning or end of the wet season and ended in the dry season. In all cases, variety Heinz was grown. Seedlings were raised on farm and sprayed once a fortnight with Dimethoate (30 ml in 15 l of water) against whiteflies. In the field, a spacing of 90 x 45 cm (Rice *et al*., 1987; Mwaule, 1995) was used. This resulted into a plant density of 50 plants per 30 m², including guard rows.

A randomized complete block design was used, and six treatments referred to as n1-n6 were applied, i.e.,

a) a tomato monocrop without spraying dimethoate, as a control treatment (n1);
b) a tomato monocrop with uprooting of TYLCV symptom-bearing plants (n2);
c) a tomato-bean intercrop without spraying dimethoate (n3);
d) a tomato monocrop with dimethoate sprayed once a week (n4);
e) a tomato monocrop with uprooting and dimethoate sprayed once a week (n5);
f) a tomato-bean intercrop with bean rows sprayed once a week with dimethoate (n6).

Treatments were planted in three blocks, and therefore treatments were replicated three times in plots of 6 m x 5 m each. Guard rows, which are normally planted with another crop that is not an alternative host e.g., maize, were established around experimental plots to minimize inter-treatment effects and also provide food to farmers. A blanket application of Mancozeb fungicide (50 g/15 l of water) was fortnightly done for all experimental treatments. The experiment was weeded regularly with a hand hoe.

5.2.3 Whitefly Population Monitoring

To keep track of whitefly population changes in the field as an alternative to turning over tomato leaves to count whiteflies present, or to using sunmica plates, vacuum collector and passive fan traps (Butler and Henneberry, 1989), cheap sticky Vaseline traps were used to trap and monitor whiteflies. Blue and yellow sticky traps (Figure 5.1), with surfaces smeared with Vaseline, were fixed in the middle and at each corner of each treatment plot to guarantee maximum trapping of whiteflies in the general tomato ecosystem. These traps were made out of used 5 l plastic jerry cans. They were modified from sticky traps reported earlier by Lewis (1973), Raccah (1986) and Green (1991). Thus, containers were cut into two pieces. Of the two pieces, the top part had a surface area of 0.015 m², whereas the bottom part had a surface area of 0.075 m² (Figure 5.1). Traps made out of top parts were used at plots' corners, while those from bottom parts formed traps fixed in the middle of plots. Blue and yellow sticky plastic traps were repeated in all treatment plots. Note was made of number of whiteflies trapped per m² every week. Counted whiteflies were removed from traps, which were then re-surfaced with fresh Vaseline to maintain stickiness.

Traps made out of 5 l plastic jerry cans trap flying whiteflies only, and were therefore used to monitor the general field whitefly population. To monitor whitefly population on individual plants per plot, another type of trap that could be used to estimate number of whiteflies infesting a tomato plant was necessary. Consequently, the Kubwa sticky trap was made out of a plastic basin and a translucent bucket with a bottom diameter of 100 cm, lower height of 45 cm, upper diameter of 25 cm, plus an upper height of 30 cm, and with a translucent top inner wall surface smeared with Vaseline (Figure 5.2).

The Kubwa trap was purposely made to trap whiteflies as an estimate of the whitefly population density on individual tomato plants. This was to take care of the fact that whiteflies rarely lay eggs on tomato leaves. Therefore, counting of nymphs could not be performed as recommended by Butler and Henneberry, (1989). Counting adult whiteflies

after turning over infested leaves is also not feasible, because many fly off before they are counted.

To get an estimate of whitefly population density per plant per plot, the Kubwa trap was inverted on one plant at a time for at least 3 minutes. Ten plants were sampled per plot. The plant stem was shaken below to induce whiteflies to fly. Whiteflies escaping to the translucent part of Kubwa trap would be trapped on the sticky surface of the bucket. Trapped whiteflies were counted and removed from the trap. Data were taken on a weekly basis starting from transplanting date.

5.2.3.1 TYLCV Incidence and Spread

Firstly, the number of plants expressing tomato yellow leaf curl symptoms was recorded at different tomato growth stages, i.e. vegetative stage, flowering stage and fruiting stage, per treatment plot. Spread of viral infection was determined in space and time by measuring the distance between old and new infections in relation to the time interval between detection of first symptoms and subsequent symptoms, in the field (Raccah, 1986). At each stage of disease spread, the number of diseased plants was recorded to develop temporal and spatial patterns of disease-spread curves. Disease spread from the first plant infected (single foci) to other plants in space was calculated using Allen's equation (Allen *et al*., 1983; Plumb and Thresh, 1983), in which distance x from the first infection source to the new infection is calculated by:

$$Px = 1\text{-}\exp(-x/x) \qquad (7),$$

whereby x is the mean distance between all new infections and their sources.
Spread in time was determined using the formula of Vanderplank, i.e. by:

$$dN/dt \qquad (8),$$

whereby N is the number of infected plants, t is the unit of time, dN is the difference between that number of plants infected, and dt is the difference in time, from when the previously infected plants were observed to the currently recorded ones. Using Vanderplank formula (7), the rate at which tomato yellow leaf curl virus *(sensu lato)* spreads in the field was established.

5.2.4 Yield Records

Mature fruits were harvested weekly and using a clock weighing scale range of (1g-10kg), yield was determined per plot. Note was taken of number of fruits harvested per treatment, total yield, marketable fruit weight and unmarketable fruit weight. Fruits were considered marketable when they had grown to maturity and had neither sign of damage nor rot.

5.2.5 Data Analysis

Data were analysed for ANOVA. Linear regression and Pearson correlations were calculated using SAS and SPSS 11.0 statistical programmes. Duncan's Multiple Range Test was also used. Sometimes General Linear Models, Least Square Means Procedure, or square root transformation was used to separate means, in case significant differences were not easy to determine. Means generated were used in Excel to develop related graphs. Standard error values (SE) were used as basis to determine variability within treatment and date effects, while F-distribution values were used to determine significance of differences between those effects (Mead and Curnow, 1990).

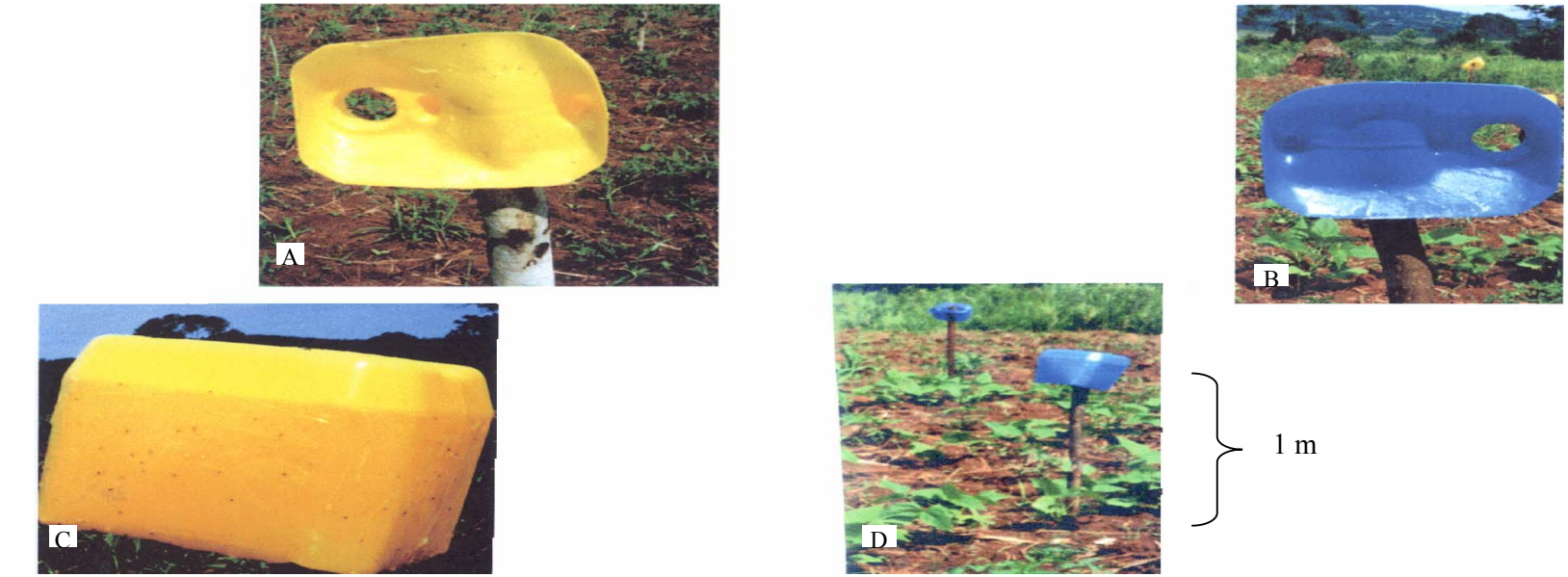
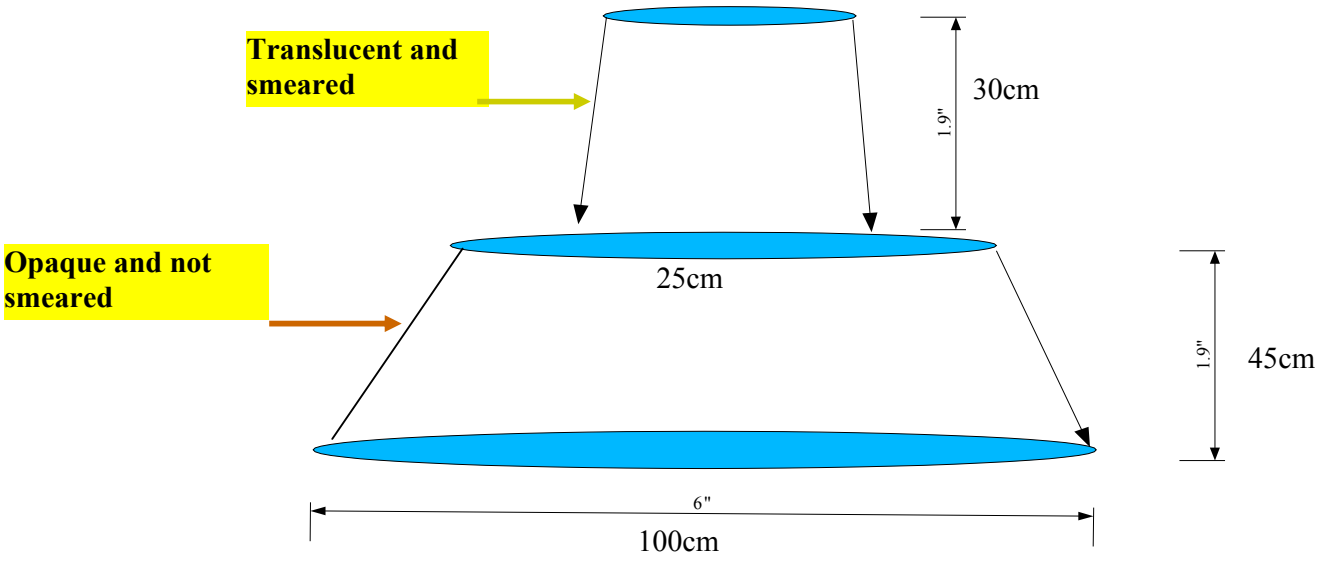


Figure 5.1: Insect traps of yellow and blue plastic material cut out of locally used containers: (A) yellow traps; and (B) blue traps, both of which were cut from the top of a 5 litre plastic jerry can, and with total inner surface area of 0.015 m ² each, which was smeared with Vaseline, and used at corners of treatment plots to trap flying whiteflies. The second set of (C) yellow traps and (D) blue traps were cut from the bottom of a 5 litre plastic jerry can. Each trap had a total surface area of 0.075 m ². They were smeared with Vaseline, and were located in every centre of treatment plots to trap whiteflies.

Figure 5.2: The Kubwa Trap, which was developed from a combination of a plastic basin and a translucent bucket, is a new sampling tool made to establish number of whiteflies present on individual tomato plants per plot. The trap has the inner surface area of the translucent part smeared with Vaseline, so that as trapped whiteflies fly towards the light, they stick onto the translucent bucket walls. Figure (A) first design without transparent section; B) improved design with transparent upper part



Fugure 5.2 C: Design of the Kubwa trap, with translucent top part and opaque lower part. This way trapped, when inverted onto a single plant, which is then agitated, whiteflies move towards light in the upper part and they are trapped against the wall smeared with Vaseline.

5.3 Results

5.3.1 General Field Weather, Whitefly Population and Tomato Yellow Leaf Curl Virus Disease Trends

In January 1999 there were 8 dry days, whereas in February 1999 there were 22 dry days. In the period January to end of February a mean average rainfall of 9.50 mm per day and a rainfall range of 0 - 16 mm per day were recorded. During trial I, March to July 1999, the amount and frequency of rainfall increased with a mean average of 10.14 mm per day and range of 0 - 69 mm per day. There were 37 dry days on a total of 122 days in this period (March to July), with 7 dry days in March, another 7 dry days in April, plus 11 more days in May and finally 12 days in June. During trial II (August to November 1999), there were 59 dry days on a total of 122 days, with a mean average rainfall of 5.70 mm per day and range of 0 - 50 mm per day. In trial III (November to February 2000), there were 51 dry days, of which 22 dry days were in February when the tomato crop had already been harvested. Apart from the period January to February 2000, when maximum temperatures reached a highest peak of 30 ºC, for the rest of the first season experiment period average ambient temperatures varied between 20 – 24 ºC. The second season was similar to the first, whereas in the third experimental season average ambient temperatures were 20 – 26 ºC (Figure 5.5b).

5.3.1.1 Whitefly Population Variation and TYLCV Incidence at Different Tomato Growth Stages

In experimental fields, an inverse relationship was observed between number of whiteflies trapped with sticky traps and number of TYLCV-symptom expressing tomato plants. At the same time, whitefly populations in the field, expressed as number of whiteflies trapped per m² of the sticky traps, went down as tomato crops grew, i.e. through vegetative, flowering, and fruiting/harvesting stages (Table 5.1). It was observed that these trends coincided with rainfall patterns. Thus, during the dry season, the whitefly population grew to a maximum average of 119 whiteflies trapped per m². In the wet season, ten weeks after transplanting, it went down to an average of

10 whiteflies trapped per m². TYLCV symptoms were more severely visible 5 weeks after whitefly population dropped. As depicted in trial I (Figure 5.3), temperature was almost constant, and its fluctuation did not seem to either influence whitefly population dynamics or affect TYLCV incidence. Similar trends were observed during trial II (Figure 5.4) and trial III (Figure 5.5 and 5.6).

Table 5.1: Mean total whitefly counts per m² for blue and yellow traps, and mean number of TYLCV infected plants, per plot in trial I (p =0.01, df = 38, F 18, 43; Pearson Correlation of R = -0.884, P = 0.01), inversely related.

Growth stages	Weeks[1] from transplanting	Ranked[2] mean whitefly counts per m²	Ranked[2] mean number of TYLCV-infected plants, per plot
Vegetative stage			
	1	48c	1d
	2	119a	0d
Flowering stage			
	3	89b	3d
	4	102ab	1d
	5	32cd	4c
	6	13de	4bc
Harvesting tage			
	7	7e	4b
	8	6e	6ab
	9	13de	6a
	10	10e	6a
	11	2e	6a
	12	1e	6a
	13	1e	6a
	14	2e	6a
	CV	0.02	0.91

[1] Week 1(13th to 20th March 1999), Week 2 (21st to 28th March 1999), Week 3 (29th March to 5th April 1999), Week 4 (6th to 13th April 1999), Week 5 (14th to 20th April 1999), Week 6 (21st to 28th April 1999), Week 7 (29th April to 5th May 1999), Week 8 (6th to 13th May 1999), Week 9 (14th to 21st May 1999), Week 10 (22nd to 29th May 1999), Week 11 (30th to 7th June 1999), Week 12 (8th to 15th June 1999), Week 13 (16th to 23rd June 1999), Week 14 (24th June to 1st July 1999). Weeks 15-20 had same trend as 13 and 14, and were not included in the table

[2] Ranking and significance are indicated by letters a-e, whereby different single letters refer to means, which are significantly different, same letters refer to means, which are not significantly different or are statistically the same

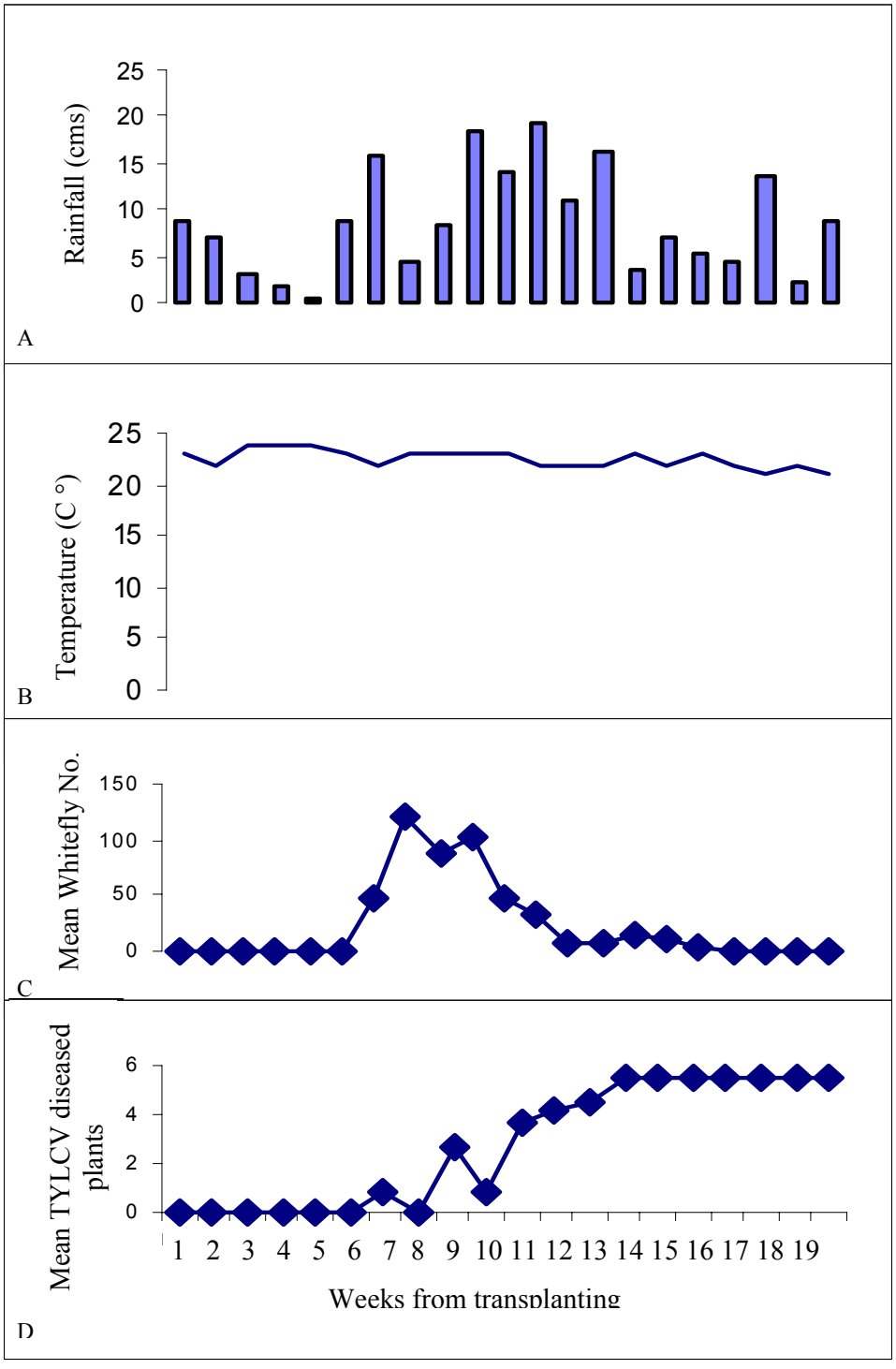


Figure 5.3: March (Week 1: 13-20th March) to July (Week 20: 6-13th Aug.), 1999 **(**A) Rainfall trends; (B) temperature trends; (C) mean number of whiteflies trapped per m^2 in trial I; (D) mean number of plants bearing TYLCV symptoms per plot in trial I

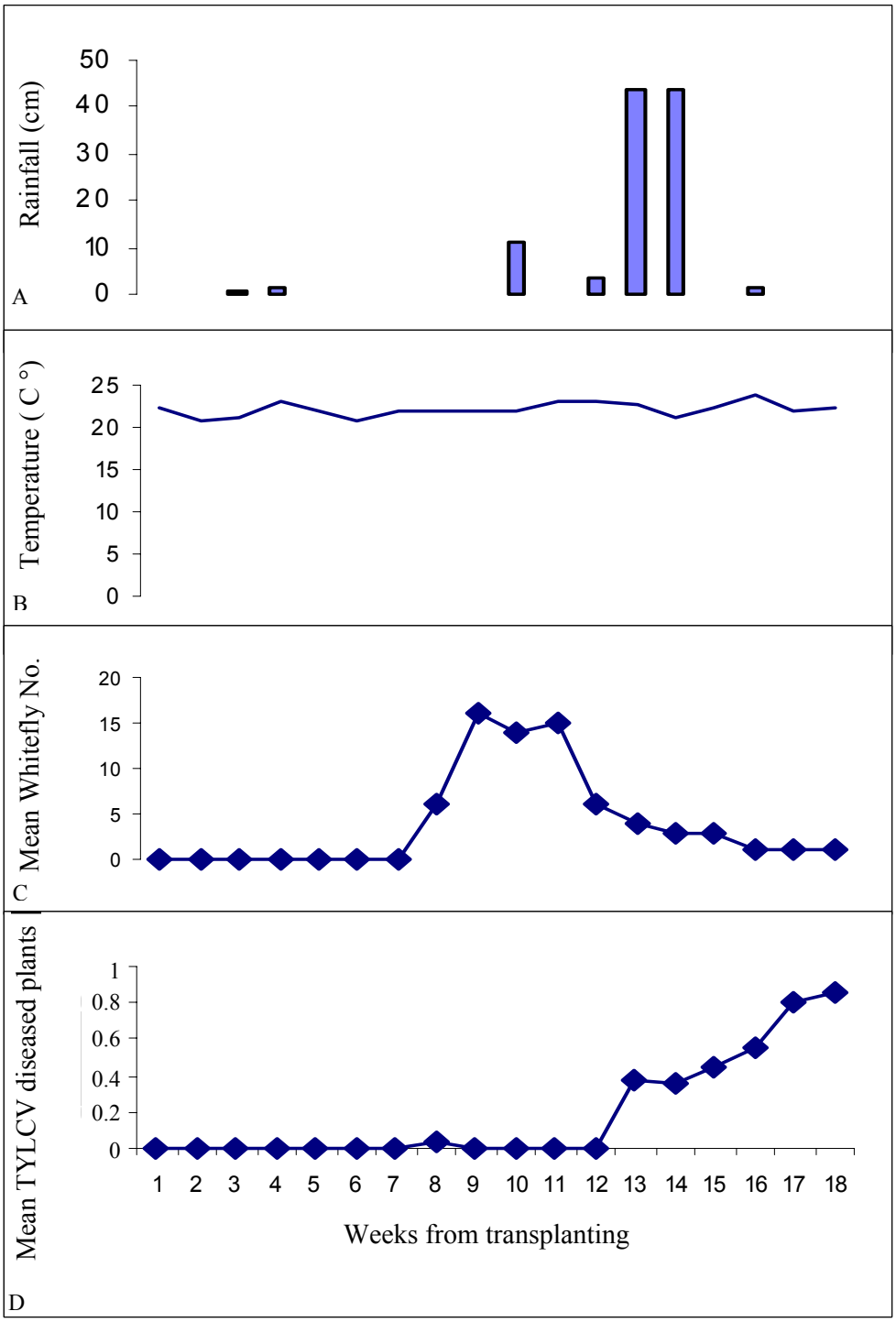


Figure 5.4: August (Week 1: 23-29th Aug.) to (Week 12: 8-14th Nov.) November, 1999[1] (A) Rainfall trends; (B) Temperature trends; (C) mean number of whiteflies trapped per m² in trial II; and (D) mean number of TYLCV diseased plants per plot in trial II. [1] During the wet season, i.e.by the 13-18th week the tomato crop had no more leaves due to *Phytophora infestans* blight disease infection, and whiteflies as well as TYLCV data collection was stopped.

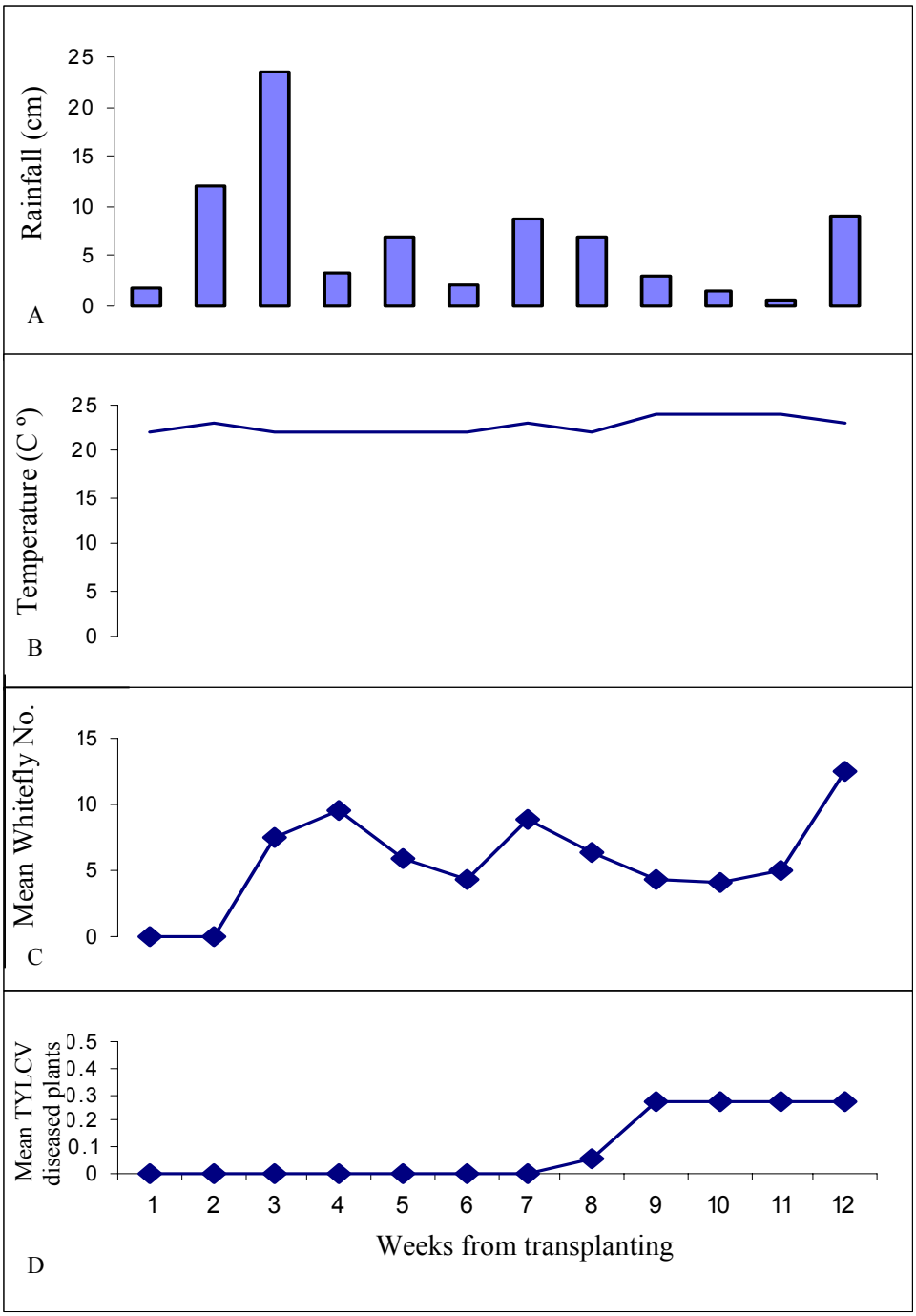


Figure 5.5: November 1999 (Week 1: 29th Nov. to 5th Dec.) – February 2000 (Week 12: 21-27th Feb.) [1] (A) Rain fall trends; (B) temperature trends; (C) mean number of whiteflies trapped per m² in trial III; and (D) mean number of plants showing TYLCV symptoms per plot in trial III

[1] Rainfall and temperature records stopped after the 12th week in February, while whitefly and TYLCV infection records continued into March 2000

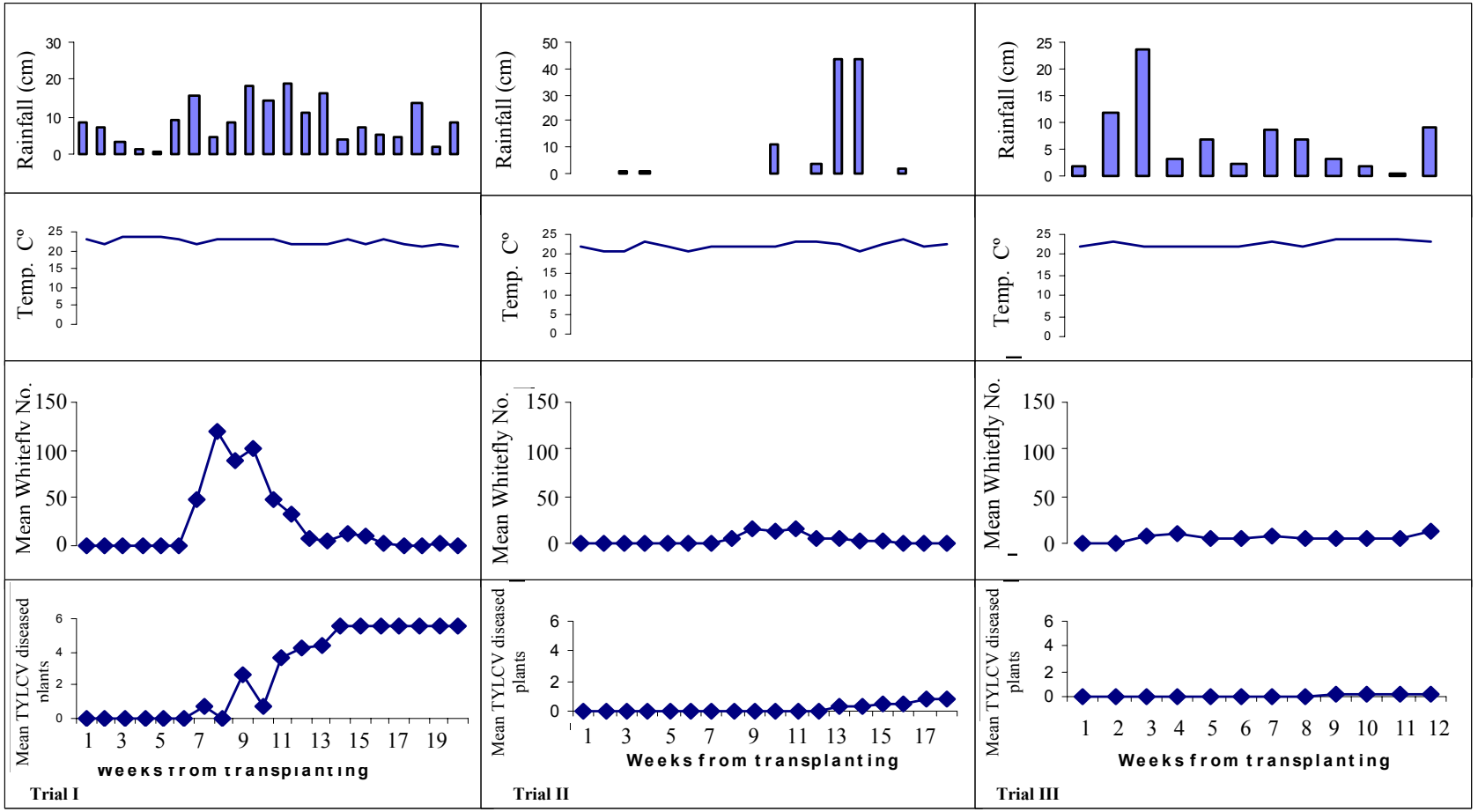


Figure 5.6: Reducing whitefly and TYLCV diseases levels from trial I to III, due to crop maturity, and increasing number of rainy days, an evidence of random extinction (Garcia-Arenal *et al*., 2000). Trial I with high levels of whitefly population and TYLCV disease incidence was preceeded by a prolonged dry season.

5.3.1.2 Whitefly Population in the Field and on Individual Plants in Relationship with TYLCV Incidence

Whitefly populations rose after the first week from 48 whiteflies trapped per m² to 119 whiteflies trapped per m² in the second week after transplanting (Table 5.1). Henceforth, there was a decline in whitefly population in the tomato experimental field throughout the remaining part of the cropping season (21st March to 1st July 1999), i.e. from 119 whiteflies trapped per m² in the second week (21st to 28th March 1999) to 1 whitefly trapped per m² in the thirteenth week (16th to 23rd June 1999) after transplanting. In contrast, the number of TYLCV-infected plants per plot went up (Table 5.1) from zero in the second week to 6 infected plants per plot in the thirteenth week. It was observed that whiteflies preferred to feed on young tender tomato to old tomato plants, which could be one of the reasons for the declining whitefly populations. However, TYLCV disease symptoms were clearly expressed only after plants had flowered. A significant negative correlation coefficient of R = -0.249, p=0.005 was recorded between the whitefly population in the field, which was recorded as number of whiteflies trapped per m² of sticky traps and number of plants bearing TYLCV symptoms between March and July 1999. At the same time, a significant negative correlationship (R = -0183, p = 0.001) was observed between number of whiteflies trapped with Kubwa trap per plant per plot and TYLCV incidence (Table 5.2). Like in the first and second planting (Table 5.3), in the third planting (Table 5.4) the number of tomato plants bearing TYLCV symptoms per plot was higher after flowering than during the vegetative growth stage, and the number of whiteflies trapped with Kubwa trap per plant per plot was negatively related with the number of plants bearing TYLCV symptoms (R = -0.0597). There was a general drop in whitefly population and TYLCV disease incidence along the experiment period (Figure 5.6). This reduction was related to the increasing number of rain days. The high TYLCV disease incidence and high whitefly population in trial I was due to the prolonged dry period that preceeded the establishment of our experiments

Table 5.2: Ranked mean weekly number of whiteflies trapped per plant per plot with Kubwa trap and related ranked mean weekly number of TYLCV symptom-bearing plants in trial I (F18, 20; df = 13, p = 0.05) (March to July 1999). Inverse relationship.

Weeks from transplanting	Ranked[1] mean whitefly count per plot	Ranked[1] mean number of TYLCV-infected plants
1	88a	3c
2	25b	4b
3	14cd	4b
4	8de	4b
5	18bc	5a
6	8e	5a
7	4e	5a
SE	0.03	0.57
CV	0.02	0.91

[1] Ranking is indicated by letters a-e, whereby for example (a) or (b) refers to means, which are significantly different, whereas (ab, cd, de, and bc) refer to means that statistically have no significant deference between them. Values with same letter are statistically the same.

Table 5.3: Ranked mean weekly number of whiteflies trapped per plant per plot with Kubwa trap in relation to ranked mean weekly number of TYLCV symptom-bearing plants (F18,20; df = 13; p = 0.001) during trial II (August to November, 1999). Inverse relationship.

Weeks from transplanting	Ranked[1]mean whitefly count per plot	Ranked[1] mean TYLCV-infection
1	1.7de	0.0c
2	5.0a	0.0c
3	4.0ab	0.0c
4	2.9bc	0.0c
5	2.8cd	0.0c
6	1.7de	0.0c
7	1.2ef	0.25b
8	0.6ef	0.39ab
9	0.4f	0.4a
SE	0.30	0.57
CV	0.02	0.91

[1] Ranking is indicated by letters a-f, whereby (a) or (b) refers to means, which are significantly different, whereas (ab, cd, de, bc, and ef) refer to means that statistically have no significant defference between them. Values with same letter ranking are statistically the same.

Table 5.4: Ranked mean weekly number of whiteflies trapped per plant per plot with Kubwa trap (f 1.25, p 0.0001) in relation to ranked mean weekly number of TYLCV symptom bearing plants (F 18,20; df = 13; p = 0.001) in trial III, (November 1999 to February 2000)

Weeks from transplanting	Ranked[1] mean whitefly count per plot	Ranked[1] mean number TYLCV infected plants
1	3.7bc	0c
2	7.4a	0c
3	1.5bc	0c
4	5.3ab	0c
5	4.3ab	0c
6	3.3bc	0.1a
7	0.4c	0.3b
8	1.8bc	0.3b
9	1.6bc	0.3b
SE	0.03	0.57
CV	0.02	0.91

[1] Ranking is indicated by letters a-c, whereby (a), (b) or (c) refers to means, which are significantly different, whereas (ab and bc) refer to means that statistically have no significant difference between them. Values with same letter ranking are statistically not different.

5.3.2 Treatment-specific Whitefly Infestation and TYLCV Incidence

With regard to the different treatment effects on whitefly infestation of tomato plants monitored using the Kubwa trap, there was no significant treatment effect on whitefly population in trial I, though plots with tomato-bean intercrop without insecticide application and those with tomato-bean intercrop combined with dimethoate had fewer whiteflies than tomato monocrop, tomato monocrop combined with uprooting, tomato monocrop combined with dimethoate, and tomato monocrop combined with uprooting and dimethoate. It was not clear whether *Phaseolus* beans in intercrop treatments could have acted as a whitefly trap crop in Trial I, II and III (Table 5.5), in which case beans would be preferred to tomato.

Furthermore, during the first week after treatment application, whitefly populations in all treatments fell to a lower level than the one recorded at the start, except in plots where tomato was intercropped with beans and bean rows were sprayed with dimethoate, in which case whitefly populations rose but later dropped like in other plots. All treatments maintained whitefly populations below three whiteflies per

tomato plant one week after treatment application. Because of persistent TYLCV transmission by whiteflies, even one whitefly would be enough to raise concern. Uprooting TYLCV-infected plants and dimethoate application treatment were able to contain whitefly populations at the lowest level throughout the experimental season, albeit with minimal difference from plots treated with tomato-bean intercrop plus spraying bean rows with dimethoate (Figure 5.7). This situation could be explained by the fact that by uprooting TYLCV disease symptom-bearing plants (single foci), which are sources of inoculum, secondary infection or polycyclic disease epidemic is eliminated. It was noted that the first planting was preceded by a very dry season, which favoured whitefly population growth, as also observed by Mazyad *et al*. (1979) and reported by Henneberry and Castle (2001). It is also worth noting that March to July rains are usually heavier than August to December rains.

For trial II (Figure 5.8), the trend was indicative of more favourable conditions for whitefly infestation. As such, treatment effect was noticeable after the second week. Furthermore, a less precise trend was observed in trial III (Figure 5.9) with limited treatment effects on whitefly populations, which could have been due to more frequent rainfall, as evidenced in Figure 5.3 and 5.6. However, within the first six weeks of November 1999 to February 2000 experimental period, whitefly populations rose drastically at two incidences, especially for the control monocrop. For monocrop plots 4 and 5 sprayed with dimethoate, the rise in whitefly populations was evident between the third to the sixth week, a situation similar to what is expected to happen when natural enemies are killed or when pests develop resistance to pesticides, but also the attractive vegetative nature of tomato plants at this stage. A similar trend was also observed, to a lesser degree, in plots where uprooting and dimethoate were applied, as well as in those with tomato-bean intercrop where bean rows were sprayed with dimethoate insecticide.

Table 5.5: Mean number of whiteflies trapped by Kubwa trap per plant per plot in trial I, II and III (df = 13; F 18, 20; p = 0.001)

Trial I (March- July 1999)		**Trial II (August – Nov. 1999)**		**Trial III (Nov. 1999 – Feb. 2000)**	
Ranked treatments	Whitefly mean count per plot	Ranked treatments	Whitefly mean count per plot	Ranked treatments	Whitefly mean count per plot
Tomato monocrop x uprooting	29	Tomato monocrop x uprooting	0.23	Intercrop x dimethoate on beans	0.21
Tomato monocrop	27	Monocrop x dimethoate	0.17	Monocrop x dimethoate	0.20
Monocrop x dimethoate	23	Intercrop x dimethoate on beans	0.17	Tomato monocrop x uprooting	0.18
Monocrop x uproot x dimethoate	22	Tomato monocrop	0.11	Tomato monocrop	0.18
Tomato-beans intercrop	20	Tomato-beans intercrop	0.04	Monocrop x uproot x dimethoate	0.18
Intercrop x dimethoate on beans	20	Monocrop x uproot x dimethoate	0.02	Tomato-beans intercrop	0.16
SE	0.30		0.30		0.30
CV	0.02		0.02		0.02

5.3.2.1 Tomato Yellow Leaf Curl Virus (*sensu lato*) Incidence and Spread under Different Experimental Treatments

5.3.2.1.1 Tomato Yellow Leaf Curl Virus Incidence

Taking all three trials, i.e. I (March-July 1999), II (August – Nov.1999), and III (Nov.1999 – February 2000), there was less TYLCV disease incidence in trial II and III than in trial I due to a dual effect consisting of treatment effects (n1-n6) in Figure 5.12, and weather changes, as shown also in figure 5.13. Thus, during trial I (Figure 5.10), disease progress curves with sigmoid-like pattern were observed for all treatment plots except for plots with tomato-bean intercrop and no dimethoate application (n3), which produced a pattern characteristic of a monocyclic disease spread (Thresh, 1998). A monocyclic disease starts off with a direct increase in number of infected plants over time, but gradually slows down and stabilizes at a certain level without rising again. From the results, it was clear that uprooting combined with dimethoate (n5), which had a significantly low disease incidence curve (Figure 5.10), was the most effective in controlling TYLCV. The same treatment (n5) also had low disease incidence in August to November 1999 (trial II) as shown in figure 5.11

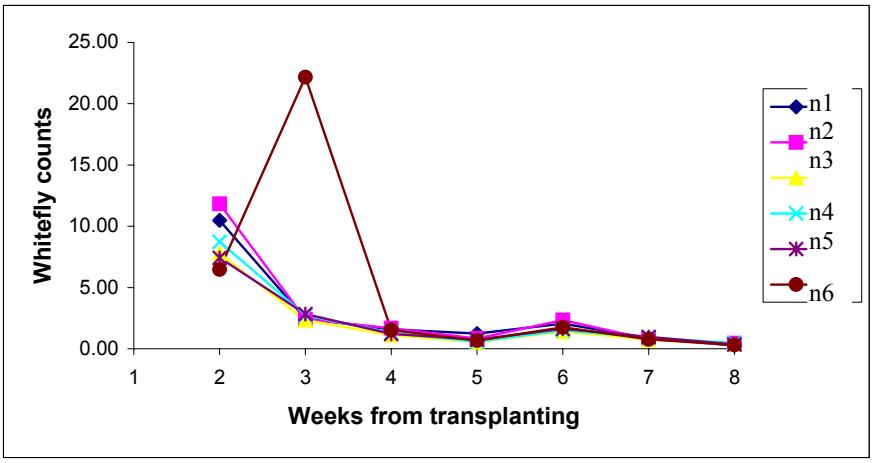


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Figure 5.7 Mean whitefly population counts per plant per plot (n1-n6) [1] per week, in trial I, Buwama, March to July 1999

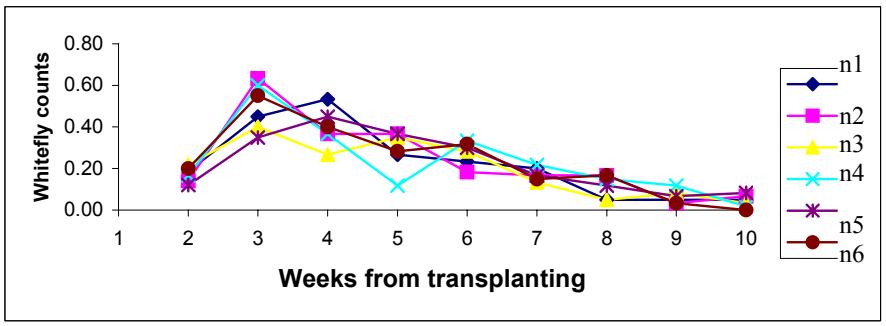

Figure 5.8 Mean whitefly population counts per plant per plot (n1-n6) [1] per week, in trial II, Buwama, August to November 1999

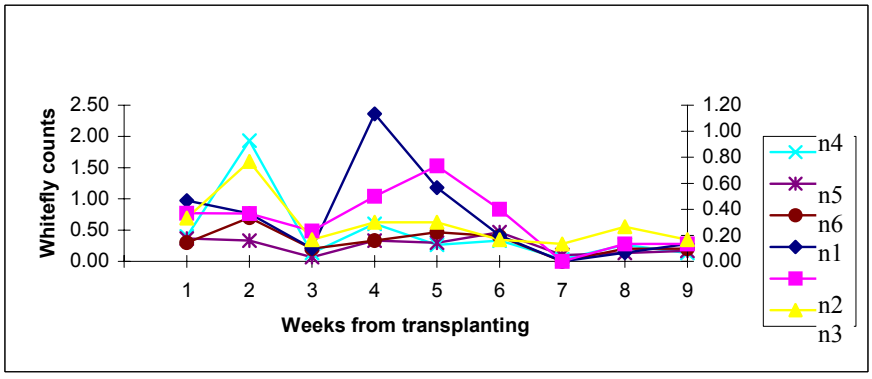

Figure 5.9 Mean whitefly population counts per plant per plot (n1-n6) [1] per week in trial III, Buwama, November 1999 to February 2000. [1]n1- Tomato monocrop without spraying dimethoate, as a control treatment; n2- tomato monocrop combined with uprooting of TYLCV symptom-bearing plants; n3- tomato-bean intercrop without spraying dimethoate; n4- tomato monocrop with dimethoate sprayed once a week; n5- tomato monocrop with uprooting and dimethoate sprayed once a week; n6- tomato-bean intercrop with bean rows sprayed once a week with dimethoate

Thus, this treatment delayed TYLCV disease onset until the 8th week, when disease incidence increased. However, the tomato crop was 8 weeks old when harvesting was carried out in the second week. During the same planting season, the tomato-bean intercrop was also effective in controlling TYLCV, but the first diseased plants occurred a week earlier than in plots where uprooting and dimethoate was applied. As in trial I, the tomato-bean intercrop with a dimethoate application only on bean rows had highest TYLCV incidence, and was therefore the least effective. Plots with uprooting only (n2) and those with a monocrop sprayed with dimethoate (n4) performed worse than the control (n1). In trial III (Figure 5.11) and after the 7th week, uprooting with dimethoate application (n5) had similar effects to those with application of dimethoate only (n4). However, in plots where only uprooting was practiced (n2), the disease was contained at zero, and therefore would serve as the best environment-friendly option to continue with after the seventh week. This time, the tomato-bean intercrop with dimethoate sprayed on bean rows had no visible TYLCV disease symptoms basically because there was a severe outbreak of late blight disease, which was due to heavy rainfall in November 1999. The latter blight probably masked TYLCV symptoms if there were any. Data collection was stopped at either the 7th or 9th week because thereafter there were no more noticeable changes, and the crop was at harvesting stages.

5.3.2.1.2 Distribution of TYLCV in Space

Uprooting and dimethoate application plots (n5) had longest mean distances between TYLCV-infected plants while the tomato-beans intercrop (n3) had shortest mean distances (Table 5.6). Calculated probabilities for the virus to spread from the initial source to a host 25 m away indicated that all treatments had the same results. There was also evidence of scattered foci of infected tomato plants in all plots.

5.3.2.1.3 Spread of TYLCV in Time

Considering overall spread of TYLCV in time, calculated following Vanderplank's formula, the highest infection rate occurred in plots where the tomato monocrop and

dimethoate were applied. Lowest infection rate of 0.032 was recorded in plots where uprooting and dimethoate were applied as indicated in table 5.7.

It was established that there was no significant difference between plots with a tomato-bean intercrop with an infection rate of 0.056 and those with tomato-bean intercrop bean rows sprayed with dimethoate (n3) with an infection rate of 0.053. The latter was also similar to that of plots with only a tomato monocrop (n1), which was also the control (0.053), and were therefore not cost-effective. At the same time, uprooting alone with an infection rate of 0.049 yielded non-significant differences from the control.

Table 5.6: Mean distance from initial infection to secondary infected plants throughout the cropping year (March 1999 to February 2000), calculated according to Allen (Plumb and Thresh, 1983)

Treatments	Ranked[1] Mean distance (cm)
Monocrop x uprooting x dimethoate (n5)	273a
Monocrop x uprooting (n2)	239ab
Tomato monocrop (n1)	230ab
Intercrop x dimethoate on beans (n6)	210ab
Monocrop x dimethoate (n4)	173ab
Tomato - bean intercrop (n3)	142b

[1] Ranking is indicated by letters a and b, whereby (a), or (b) refers to means, which are significantly different, whereas (ab) refer to means that statistically have no significant difference

Table 5.7: Infection rate for the different experiment plots for the period March 1999 to February 2000, calculated according to Vanderplank formula (dN/dt)

Treatments	Ranked[1]annual mean infection rate (dN/dt)
Monocrop x dimethoate (n4)	0.074a
Intercrop x dimethoate on beans (n6)	0.056ab
Tomato - beans intercrop (n3)	0.053ab
Tomato monocrop (n1)	0.053ab
Monocrop x uprooting (n2)	0.049ab
Monocrop x uprooting x dimethoate (n5)	0.032b

[1] Ranking is indicated by letters a and b, whereby (a), or (b) refers to means, which are significantly different, whereas (ab) refer to means that statistically have no significant difference

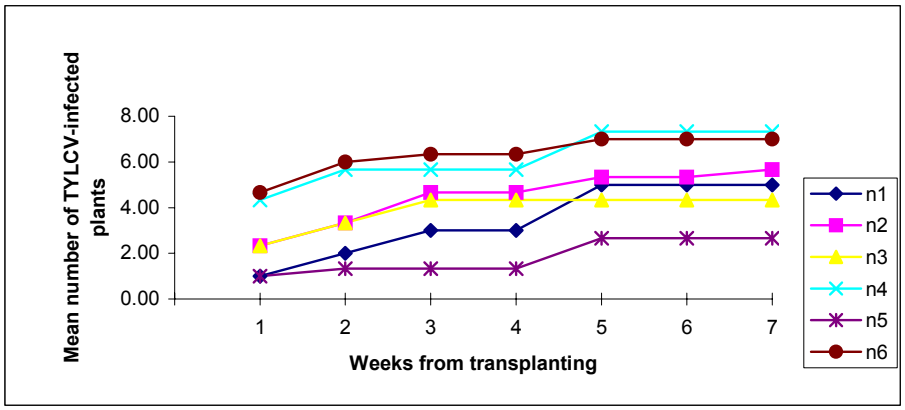

Figure 5.10: TYLCV progress curves for each treatment (n1-n6) [1] in trial I, Buwama, March to July 1999

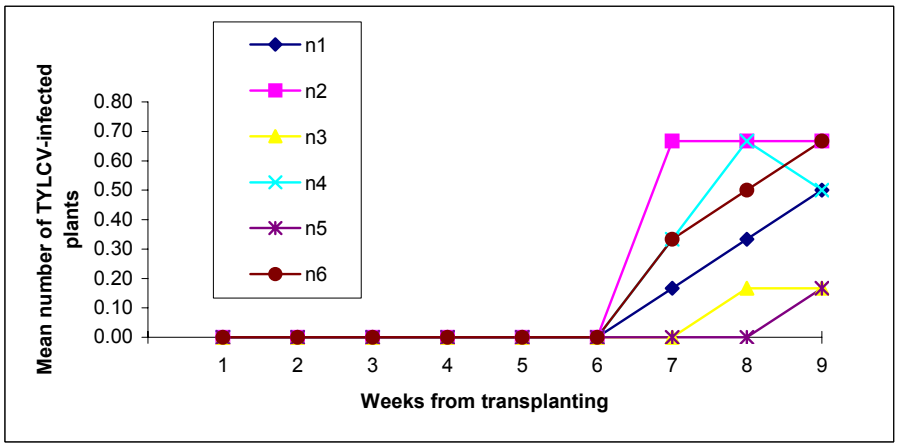

Figure 5.11: TYLCV progress curves for each treatment (n1-n6) [1] in trial II, Buwama, August to November 1999

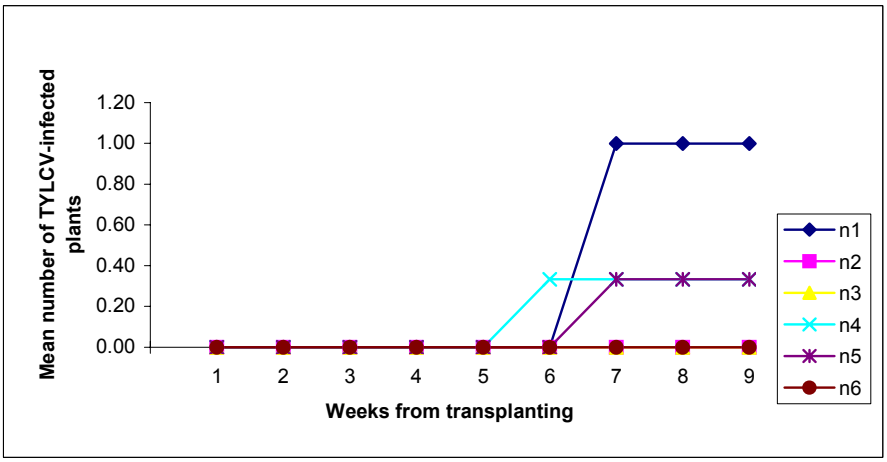

Figure 5.12: TYLCV progress curves for each treatment (n1-n6) [1] in trial III, Buwama, November 1999to February 2000. [1]n1- Tomato monocrop without spraying dimethoate, as a control treatment; n2- tomato monocrop combined with uprooting of TYLCV symptom-bearing plants; n3- tomato-bean intercrop without spraying dimethoate; n4- tomato monocrop with dimethoate sprayed once a week; n5- tomato monocrop with uprooting and dimethoate sprayed once a week; n6- tomato-bean intercrop with bean rows sprayed once a week with dimethoate.

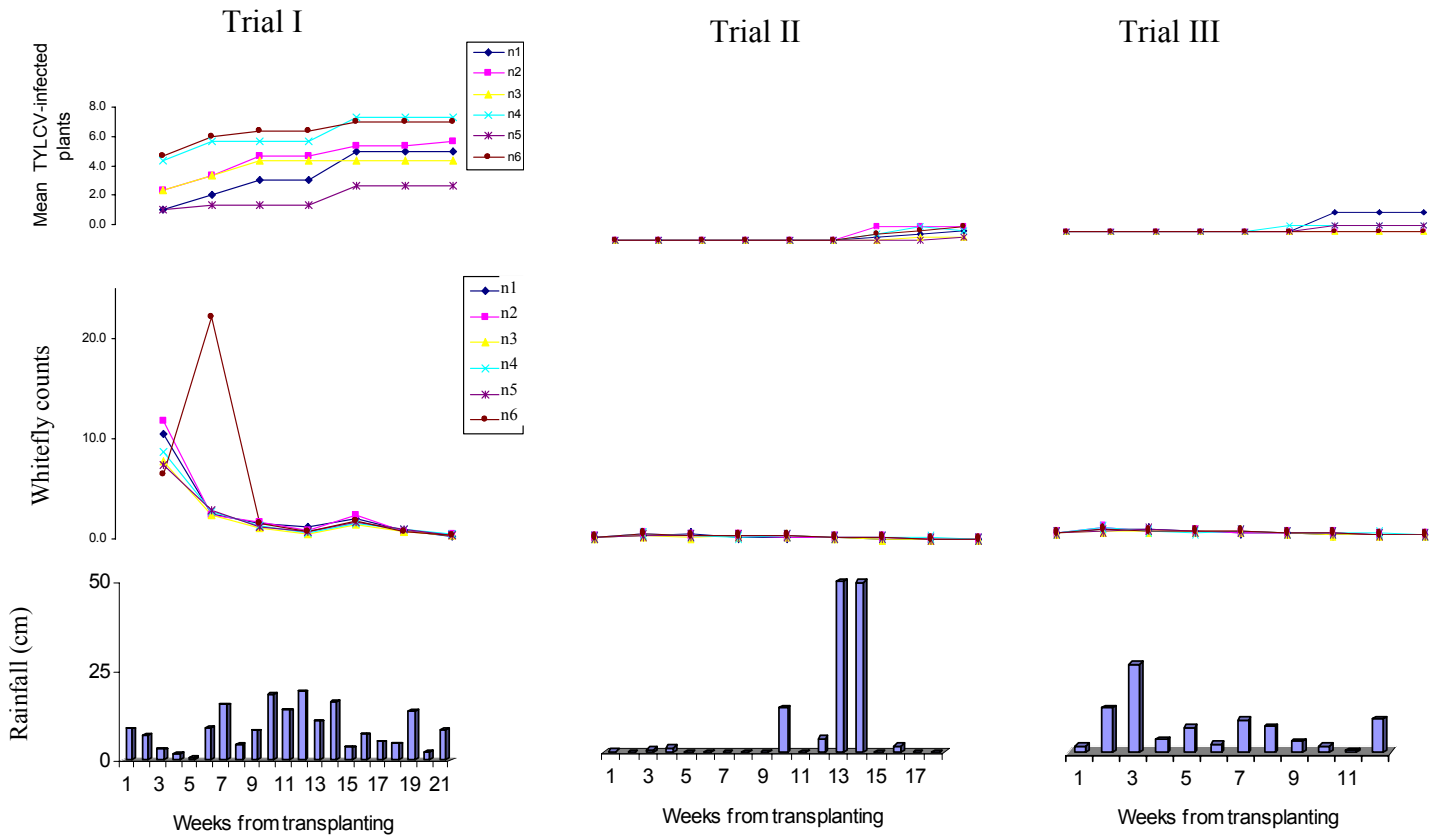

Figure 5.13: Treatment effects on incidence of TYLCV diseases and whitefly infestation of tomato plants in the three trials, i.e. Trial I, Trial 2 and Trial 3, in relation to rainfall. Like in the general study of whitefly population dynamics in the tomato agro-ecosystem (sections 20.1.1 and 20.1.2), rainfall frequency during the experimental period reduced both number of whitefly on tomato plants and number of TYLCV diseased plants in individual treatments. However, being that whiteflies transmit TYLCV in a persistent maner, even one whitefly in the field is of paramount importance. Similary, a single focus of TYLCV disease is a potential source of inoculum for other plants to be infected, and it is worth when few infected plants represent scattered foci from where the disease can be transmitted to other plants, not to mention the role played by alternative hosts.

5.3.2.2 Treatment Effect on Tomato Productivity

Tomato yield differed between treatments in trial I. Tomato monocrop combined with dimethoate application (n4), and uprooting combined with dimethoate (n5) had the biggest number of fruits harvested. However, uprooting combined with dimethoate (n5) had low TYLCV infection. At the same time, bean-tomato intercrop with dimethoate sprayed only on beans (n6) had lowest number of tomato fruits (Table 5.8). The control treatment with only a tomato monocrop (n1) had more fruits than plots where uprooting was applied, and those with tomato-bean intercrop. Total yield and marketable yield results were directly related to number of fruits harvested as evidenced in table 5.8.

Taking the number of tomato fruits produced per treatment in trial II, a trend similar to that observed in trial I was noted. Tomato monocrop combined with dimethoate plots (n4) and those with uprooting combined with dimethoate (n5) had the biggest number of fruits. They were also significantly different from monocrop plots with uprooting (n2), plots with tomato-bean intercrop (n3), and from those plots with tomato-beans intercrop combined with dimethoate (n6). Furthermore, tomato-beans intercrop with dimethoate applied on bean rows alone (n6), which had lowest number of fruits (Table 5.8), was also found to have the highest number of plants infected with TYLCV. This implied that intercropping with beans does not reduce TYLCV infection. TYLCV is transmitted in a persistent manner. Therefore and if at all, beans attracting whiteflies to the intercrop plot could have exposed tomato plants to more chances of infection from even short interval feeding by TYLCV inoculum-bearing whiteflies. Uprooting with dimethoate was therefore still the most effective treatment, while uprooting, tomato-bean intercrop and tomato-bean intercrop combined with dimethoate, which gave yields lower than that of control plots, were not effective. Apart from tomato-bean intercrop combined with dimethoate on beans only (n6), all dimethoate-treated plots had on average better total yields than those without. Thus, n4 had total yield of 5.7 kgs in trial I, 14.8 kgs in trial II, and 1.6 kgs in trial III, while n5 had 4.9 kgs, 14.8 kgs, and 1.1 kg respectively, as indicated in table 5.8. In terms of weight and marketable quality, tomato monocrop combined with dimethoate (n4), uprooting combined with dimethoate (n5), and tomato-bean intercrop with dimethoate

on bean rows only (n6), still performed better than those plots without dimethoate treatment. At the same time, plots with tomato-bean intercrop combined with dimethoate on bean rows (n6) had few, but heavy and market-quality fruits. Uprooting alone gave better results than the control, even though the difference was not significant. The bean-tomato intercrop without dimethoate (n3) performed worst. There was also a negative relationship of $R = -0.1438$, $p = 0.0402$ between number of plants infected with TYLCV and percentage marketable yield.

Yield results in trial III were different from those in trials I, and II. Thus, there was no significant difference between fruit numbers per treatment (Table 5.8). Data on fruit weights indicated that intercropping tomato with beans and applying dimethoate on bean rows (n6) gave lowest total yield followed by uprooting combined with dimethoate (n5), which was not significantly different from either control (n1), uprooting alone (n2), tomato-bean intercrop (n3), and tomato monocrop combined with dimethoate (n4). For marketable yield, the tomato-bean intercrop combined with dimethoate (n6) had the lowest marketable yield followed by uprooting alone (n2).

Table 5.8: Ranked (a-c) [1] mean number of fruits, total yield and marketable yield (kg) for three replicate plots put together (90 m²), for each treatment in trials I, II and III

Treatments	**Trial I (March - July, 1999)**			**Trial II (Aug. – Nov. 1999)**			**Trial III (Nov. 1999 – Feb. 2000)**		
	Number of fruits per plot	Total yield (kg)	Marketable yield (kg)	Number of fruits per plot	Total yield (kg)	Marketable yield (kg)	Number of fruits per plot	Total yield (kg)	Marketable yield (kg)
1-Monocrop control	73ab	4.1ab	3.6ab	119b	10.8bc	10.7ab	26a	1.5a	1.2a
2-Uprooting	59bc	3.5bc	3.2ab	119b	12.2b	11.4ab	24a	1.6a	0.59b
3-Tomato/beans intercrop	40c	2.2c	1.9c	106c	10.1bc	9.5c	26a	1.9a	1.6a
4-Monocrop and Dimethoate	93a	5.7a	5.1a	139a	14.8a	13.9a	24a	1.6a	1.4a
5-Uprooting and Dimethoate	89a	4.9ab	4.6ab	142a	14.8a	13.7a	21a	1.1ab	0.8ab
6-Tomato/beans intercrop and dimethoate on beans only	34c	1.9c	1.7c	112bc	14.5a	13.7a	11b	0.5b	0.5b
MSE	2.8	9.9	9.9	57.9	7.6	7.2	28.5	1.5	1.2

[1] Ranking is indicated by letters a-c, whereby (a), (b) or (c) refers to means, which are significantly different, whereas (ab and bc) refers to means that statistically have no significant difference

5.4 Discussion

The study confirmed that weather changes influence whitefly population dynamics and TYLCV incidence in Uganda. It was also confirmed that whatever the weather condition, whitefly infestation was more common on young tomato plants with fresh and soft leaves than on old tomato plants. However, it was observed that TYLCV symptoms were visible at an advanced stage of tomato growth, except for cases of very early seedling infection, quasi-primary infection.

Our experiments were replicated during wet and dry seasons to take care of natural conditions like rainfall and temperature, which affected our trials in addition to experimental treatments. Riley *et al.* (1995) and Elkiton (1993) observed the effect of climatic factors on whitefly populations and specifically reported rainfall, relative humidity, temperature and wind to induce increase or reduction in whitefly populations. However, during our study temperature was almost constant throughout the year, whereas rainy weather conditions varied and also caused a fall in whitefly populations (Figures 5.3-5.5). Thus, in May to June and October to December rainy seasons, which were associated with severe TYLCV symptoms, whitefly populations were low (Figures 5.3-5.5). Because of the bi-modal pattern of rainfall, two periods of low whitefly populations occurred in alternation with high whitefly population periods. Moustafa (1991) found similar weather effects on TYLCV incidence and on whitefly populations in Egypt. He reported low TYLCV incidence in Spring and early Summer (February to April) and high incidence at the end of Summer (September to mid-October). In Tanzania, Nono-Womdim *et al*. (1996) reported TYLCV disease symptoms and a high whitefly population to occur at the same time, i.e. from November to February. This situation differs from that found in Uganda probably because of irrigated tomato production that takes place most of the year, especially in Arusha region of Tanzania. Likewise our findings were contrary to Mansour *et al*. (1992) and Mehta *et al*. (1994) reports, which indicate that high incidence of TYLCV coincides with high whitefly populations. The latter authors together with Cohen and Antignus (1994) reported that TYLCV occurrence and spread are directly proportional to whitefly populations. We, however, found that there was a time lag of 3-4

weeks between first observation of whiteflies on tomato and symptom expression. As a result, there are more TYLCV (*sensu lato*) disease symptoms recorded on old tomato plants than on young plants during the wet season, as shown in table 5.1.

Furthermore, it was established that TYLCV disease seems to spread in a monocyclic manner. In this pattern, tomato plants are infected without subsequent spread of the disease. Our study also showed presence of scattered foci of infected tomato plants, i.e. isolated diseased plants, in all treatments (Table 5.6). It was not possible to establish whether these new infections are due to whiteflies coming in from outside (external host to tomato) or by whiteflies moving from on infected tomato plant to another within the field (tomato to tomato disease spread), which would then result into a polycyclic disease spread pattern. To manage TYLCV diseases, timely planting is considered a potential solution based on response to weather changes by whitefly vector populations and TYLCV symptom expression, as mentioned above. Sticky Vaseline traps showed higher whitefly populations in the young vegetative crop followed by the flowering crop, whereas few were trapped from the crop at harvesting stage (Table 5.1 and Figures 5.3c, 5.4c, 5.5c). This result implies that early application of uprooting combined with dimethoate (n5) is most effective for both whitefly management and TYLCV control. The combination was better than either uprooting or dimethoate application alone, especially in trial II, as shown in table 5.5 and figures 5.9 – 5.11. Perring *et al*. (1999) reported that integrated virus control was more effective than applying one option. Brown (1997) review explained that whiteflies multiply faster on TYLCV infected plants. This being true, uprooting is advantageous because it deprives whiteflies of the suitable environment for rapid multiplication.

Experimental treatments with a bean intercrop produced very low tomato fruit yields, and yet had low whitefly population. Investigation of other causes was beyond the scope of this study, but it requires future consideration as to whether there is competition for nutrients between these two crops. At the same time, non-significant differences between treatments (n1, n3, n4 and n6) in trials I –III were recorded, and this was attributed to such factors as prevailing

winds and alternative hosts, which may have influenced whitefly population in all treatments, and hence affected TYLCV incidence as well as general treatment performance.

More whiteflies were observed on the bean crop than on tomato within the intercrop. This way the bean intercrop was considered able to influence whitefly population dynamics by acting as a trap crop, the so-called "pull effect". In treatment 6 (Table 5.5), beans attracted whiteflies whereas dimethoate was applied to kill attracted whiteflies. This can be termed a pull and kill method, but it was not significantly effective in reducing whitefly populations. Application of dimethoate on beans may also have repelled whiteflies from beans to un-sprayed tomato rows. This would explain the high number of whiteflies trapped on individual tomato plants using Kubwa trap in this treatment (Table 5.5). A similar situation may arise when farmers apply pesticides on their tomatoes, thus causing whiteflies to migrate from sprayed to unsprayed hosts in the complex agro-ecosystem (Annex 3).

Furthermore, spraying a tomato monocrop with dimethoate insecticide, which is a broad-spectrum and systemic insecticide, is similar to the situation in Ugandan farmers' fields, where continuous application of various pesticides takes place. A monocrop sprayed with dimethoate (n4) performed worse than the control plot (n1). Continuous application of such insecticides may be leading to eradication of whitefly natural enemies (Henneberry and Castle, 2001; Riley *et al*., 1995; Duffus, 1995). Uprooting alone (n2) gave better results than where dimethoate was applied (n4) without uprooting diseased plants. Uprooting is a better option if farmers are to manage TYLCV disease, but avoid destroying whitefly natural enemies. Therefore, future studies need to focus on identifying whitefly natural enemies and designing environment-friendly whitefly control methods such as use of virus resistant tomato varieties, use of mulches, and uprooting infected tomato plants.

Another control measure for whitefly and TYLCV generated from this study is a shift in planting date by utilising periods when whitefly populations are low. During our study, it was established that whitefly populations were low in May to June and October to November 1999. This may be variable over the years, and therefore continuous studies would be required

to generate data for purposes of modelling whitefly population dynamics. As for now, initiation of nursery beds during whitefly-free periods would help to avoid primary TYLCV infection. The objective is to have mature plants with hardened leaves, which are not palatable to whiteflies by the time whitefly population rises again. Related non-pesticide methods for the control of whiteflies, such as use of plastic mulches, were found to be effective in the Middle East (Ioannu *et al*., 1991). Whereas our study considered several factors involved in influencing whitefly transmission of TYLCV, it is suggested that a systemwide /holistic approach to studying tomato yellow leaf curl viruses be applied in future studies on this subject. In conclusion, as observed during our study, rainfall patterns, uprooting, intercropping, and use of dimethoate insecticide influence whitefly populations, and hence influence TYLCV disease incidence.

At the same time, we cannot overlook the fact that tomatoes are grown by smallholder farmers, and in varying and complex agro-ecosystems, which are characterized by a rich biodiversity of crops (Annex 3). This situation enhances TYLCV transmission. Our study identified weed species associated with the tomato agro-ecosystem to be *Ageratum conyzoides, Commelina benghalensis*, *Conyza floribunda*, *Crassocephalum* spp., *Desmodium* spp., *Euphorbia heterophylla, Physalis floridana* and *Imperata cylindrica.* At least three weeds, i.e. *Euphorbia heterophylla*, *Physalis floridana* and *Desmodium* spp. (Table 3.13), were confirmed to be TYLCV-UG alternative hosts. The existence of a monocyclic pattern of TYLCV disease could imply that it was transmitted from weeds to tomato in the experimental field, and no subsequent spread took place from tomato to tomato within the experiment. TYLCV transmission from weeds to tomato was also reported in Tanzania (Nono-Womdim *et al*., 1996) and in the Middle East (Ioannou *et al*., 1991). On the other hand, Caciagli *et al*. (1995) reported a polycyclic pattern of TYLCV transmission from tomato to tomato by *B. tabaci.* Furthermore, beans, sweet potato and cassava found in the farming system, and as reported by Legg (1996), are alternative hosts of whiteflies. Consequently, we can conclude that timely weeding is also important in management of tomato yellow leaf curl diseases (*sensu lato*), in addition to running a proper crop rotation. Alternative crop hosts could play a big role in offering breeding grounds to tomato infesting whiteflies. It is important that future

research establishes whether whiteflies observed on other crop hosts are vectors of tomato yellow leaf curl diseases. Even though this information is not available in Uganda, use of these whitefly and tomato yellow leaf curl viruses alternative host crops as tomato intercrops should be discouraged, and instead crop rotation is recommended. Something, which may be difficult in a situation where smallholder farmers have scarce land and grow crops like cassava and sweet potato for food security (Annex 3).

From another school of thought, whiteflies immigrations may also influence the effectiveness of whitefly management treatments, as was also observed by Cohen and Antignus (1994) in Jordan Valley and Simone and Short (1998) in Florida. According to our study, general whitefly population evolutions in the tomato field environment had the same trends, whether monitored with either sticky traps or Kubwa trap (Figures 5.1-5.2), even though they differed in magnitude. While yellow and blue sticky traps targeted whiteflies in the overall experiment environment, the Kubwa trap targeted whiteflies resting on tomato plants. Thus, absolute field populations of *B*. *tabaci* are not easy to determine (Gerling and Mayer, 1995), any sticky traps would measure migrating whiteflies as well as those in trivial flights, (Cohen *et al*., 1988), defined as those movements, which cover very short distances within and between plants. This is the case with yellow and blue sticky traps, which are fully exposed to the atmosphere. However, these traps are cheap, made of used plastic containers, and therefore affordable by farmers. Therefore, tomato farmers in Uganda can use these sticky traps as monitoring tools for whiteflies before making a decision to apply pesticides.

Changes in weather conditions influence tomato growing seasons and whitefly populations, which in turn influences incidence of tomato yellow leaf curl disease. Similarly, farmers' activities like planting date, weeding, and pesticide applications affect whitefly populations and tomato yellow leaf curl diseases incidence, as well as tomato fruit yield. Because of the variable treatments used in our experimental trials, control plots, and continuous planting, irrespective of weather conditions, our trials on average recorded lower yields than the reported average yield for Uganda (10 ton/ha), whereby farmers go for only optimum production periods of the year. Farmers apply excessive pesticides irrespective of pest

population levels, in which case other pests that affected yields in our trials, like *Helicoverpa armigera* (African ballworm), are completely wiped out in farmers' trials (Annex 3).

Therefore, we can deduce that our study proved the null hypotheses to be true, and to have achieved set objectives. This way useful information, which can be used to generate appropriate whitefly and TYLCV management packages, is made available and future research gaps established.

CHAPTER 6

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

In this chapter, we discus information generated in chapters 3-5, in relation with the need to develop environment-friendly and sustainable tomato virus disease management packages for tomato growers, as well as gaps that require future research attention.

6 CHAPTER 6

General Discussion, Conclusions and Recommendations

6.1 General Discussion

From our results, we conclude that our findings concur with hypotheses formulated at the onset of the study. Several viruses, including those already reported in East Africa, namely AMV, ChiVMV, CMV, PVMV, PVX, PVY, ToMV, ToLCV, TSWV, and TYLCV (CMI, 1969; Yassin, 1989; Nono-Womdim *et al*., 1996; Czosnek and Laterrot, 1997), infect tomato in Uganda. Tomato leaf curl viruses are amongst major viruses whose incidence and spread are influenced by presence of the whitefly vector (*Bemisia tabaci*) (Moriones and Navas-Castillo, 2000; Anderson and Morales, 2005), and weather conditions within the agro-ecosystem. Specific findings and conclusions include what follows below.

A wide range of weeds and other crops were observed to occur in both tomato fields and surrounding ecosystem (Annex 3). Most weeds had been reported in earlier studies to be alternative hosts to tomato viruses (Tables 3.4 and 3.5). Our study also established that weeds could act as alternative hosts to some tomato viruses that were identified during this study (Table 3.12). This has led to a clear understanding of the necessity for timely weeding of tomato fields as an option for virus disease management. Furthermore, the complex nature of the agro-ecosystem, within which a majority of tomato farmers work, requires a holistic approach to future studying of tomato virus pathosystems (Anderson and Morales, 2005). The majority of Ugandan tomato growers are smallholder farmers. They try to avoid risks related to farming by growing a multiplicity of crops (Annex 3), which contributes to creating a complex agro-ecosystem.

Within this complex system, the tomato crop is exposed to numerous pests and diseases (Annex 3). The study revealed that a number of viruses infect Ugandan tomato, and cause virus diseases, which are characterized by symptoms such as leaf curl, mosaic and mottling.

These viral disease symptoms were not specific to a particular virus. For example, combinations of two to five viruses were encountered in individual tomato plants. In one experiment, it was observed that PVMV alone caused no visible significant damage to the crop, but co-existence with ChiVMV in the same plant showed severe and synergistic effects on tomato crop performance.

Viruses responsible for those symptoms observed on tomato were reported for the first time, in Uganda, i.e. RNA viruses ChiVMV, PVMV, AMV, CMV, TSWV, PVY and PVX, as well as begomoviruses ToLCV-UG and TYLCV-UG (Chapter 3). However, tomato plants infected with the latter two DNA viruses had characteristic small leaves, with marginal yellowing and leaf curl symptoms. ToLCV-UG seems to be a new begomovirus different from tomato yellow leaf curl viruses (*sensu lato*) found elsewhere in the New and Old Worlds. Whereas this finding was based on intergenic sequence comparison results (Padidam *et al*., 1995), later it was confirmed in our other research experiments conducted in collaboration with Dr. Green of the World Vegetable Center, Taiwan (Shih *et al*., 2005), which were based on a complete DNA sequence as was recently recommended by ICTV (Fauquet *et al*., 2003).

Field studies of the virus-vector relationship established that virus occurrence varied in space and time, and with management practices, crop development stage, and weather conditions. A negative relationship ($R = -0.14$, $p = 0.04$) was established between number of plants infected with TYLCV (*sensu lato*) and percentage marketable tomato yield. On the other hand, *Bemisia tabaci*, the vector of TYLCV *(sensu lato)* showed a variable population, which depended on micro-climatic conditions in the agro-ecosystem, with high populations prevailing during the dry season and decreasing with the onset of rain, and in turn influenced tomato yellow leaf curl virus disease incidence. The more mature the tomato crop, the less it was infested with whiteflies ($R = -0.5$, $p < 0.0001$), for whiteflies prefer tender leaves, which are found on young tomato plants (Nono-Womdim *et al*., 1996). Therefore, variation in date of planting could be used in management of both whiteflies and TYLCV (*sensu lato*). Furthermore, an integrated package of uprooting TYLCV disease symptom bearing plants and application of the insecticide dimethoate was found to be the most effective of the six

treatments applied in reducing whitefly populations and controlling TYLCV (*sensu lato*). Perring *et al*. (1999), while considering the effect of epidemiological factors and transmission of insect-vectored viruses on the effectiveness of chemical treatments, found that the best approach to vector and virus disease management was to use more than one control measure. Treatments applied during our study indicated that uprooting combined with application of dimethoate was the most effective control. Chan and Jeger (1994) reported that uprooting was more effective especially when plants are sparsely planted. Tomato is densely planted and canopies overlap. Even though, our finding indicated that at low disease incidence both chemical and uprooting were individually effective. Vaseline-smeared sticky traps made from locally available 5 litre yellow plastic jerry cans, were used to monitor infestation. They trapped an average of 100 whiteflies per 1m², and had efficiency either of 483, 100 or 117 whiteflies per 1m² for the first, second, and third planting experiment, respectively.

6.2 Conclusions and Recommendations

These results present a good starting point for tomato virus diseases diagnosis in Uganda; throw more light on the use of partial sequences to compare geminiviruses; and give sound tomato yellow leaf curl virus disease and vector management options. Consequently, our findings concur with hypotheses formulated at the onset of the study, and the following is the summary of our key conclusions and recommendations:

Several viruses, including those already reported in East Africa, infect tomato in Uganda.
A number of viruses infect Ugandan tomato, and cause virus diseases, which are characterized by symptoms such as leaf curl, mosaic and mottling. These viral disease symptoms are not specific to a particular virus.
Viruses responsible for a number of leaf curl, mosaic and mottling virus symptoms observed on tomato were reported for the first time, in Uganda, i.e. RNA viruses ChiVMV, PVMV, AMV, CMV, TSWV, PVY and PVX, as well as begomoviruses, i.e. ToLCV-UG and TYLCV-UG. Basing on the genetic composition of its replication gene and coat protein gene, and to the best of our knowledge, ToLCV-UG is a new virus reported for the first time in

Uganda. It is also the first time to report ChiVMV on tomato in Uganda and East Africa at large.

We established that weeds act as alternative hosts to some tomato viruses identified, which lead to a clear understanding of the necessity for timely weeding of tomato fields as an option for virus disease management. *Occimum basilica* was reported here to be an alternative host of ChiVMV and PVMV for the first time.

Combinations of two to five viruses exist in individual tomato plants. These mixtures contribute to the severity of tomato virus diseases in Uganda.

Tomato yellow leaf curl viruses are amongst major viruses whose incidence and spread are influenced by presence of the whitefly vector (*Bemisia tabaci*), and weather conditions within the agro-ecosystem in Uganda.

We found that whiteflies preferred young tomato plants to old plants. It is recommended that whiteflies be controlled at nursery stage before transplanting seedlings, and before infection or when vector and disease levels are still very low. As reported earlier, tomato yellow leaf curl virus infection at an early tomato plant development stage results into death before fruiting or only single trush setting, as also was observed by Yassin (1982).

Uprooting diseased plants and applying dimethoate was an effective control for whitefly vectors. Tomato growers are advised to apply this integrated management package for TYLCV (*sensu lato*) and the whitefly vector, especially if done during the dry season and the first part of the rainy season when whitefly populations are high. Even though there is trans-progeny transmission of TYLCV (*sensu lato*), which is also persistent in viruliferous whiteflies (Rubinstein *et al*., 1999), in this way, farmer expenditure on pesticides would be minimized and the amount of pesticides filtering through to the environment would be tremendously reduced.

Whiteflies are one such vector observed on tomato and known to be responsible for transmitting tomato yellow leaf curl virus diseases (Anderson and Morales, 2005). Whitefly adults are not easy to count because they fly away immediately when their resting ground is disturbed. Therefore, for sampling from individual tomato plants, the Kubwa sticky trap and yellow sticky jerry can traps would serve as new formats of cheap sticky traps (Chapter 5). Ugandan tomato growers could easily get these cheap traps and use them as whitefly monitoring tools before deciding to spray, in order to minimize amount of pesticides applied to the environment. In this way, tomato production costs would also be reduced. Therefore, these sticky traps are recommended for further evaluation in comparison with other known sampling tools.

Our study results provide a firm basis for future research on tomato viruses in Uganda. The complex farming system and the multiplicity of viruses identified on tomato, imply possible complex involvement of vectors observed during the preliminary survey (Annex 3). Hence, the need for more research geared towards understanding the role of insect pests, as virus vectors, and possible control measures, which would provide smallholder farmers with appropriate tomato virus management tools.

6.2.1 Future Research Proposals

Having documented major viruses infecting tomato, identified the major causal organisms of yellow leaf curl symptoms and their vector, as well as having established appropriate integrated management practices, future research efforts should focus on the following proposals:

- conduct a countrywide (62 more districts) survey of tomato viruses;
- investigate further the biodiversity, incidence and mode of transmission of ChiVMV in Uganda;
- investigate the occurrence of TYLCV-UG and ToLCV-UG in other districts of Uganda, while using recently developed cheaper molecular techniques like multiplex

PCR (Potter *et al*., 2003; Gorsane *et al*., 2005), and loop-mediated isothermal amplification reaction (LAMP) (Fukuta *et al*., 2003);

- evaluate currently grown tomato varieties for resistance to TYLCV-UG and ToLCV-UG; and investigate possibilities for generation and use of transgenic locally marketable tomato varieties with resistance to tomato leaf curl viruses; and
- study the biodiversity and molecular relationship between whiteflies observed on tomato and those found on other plants within the agro-ecosystem.

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8 ANNEXES

Pages

Annex 1:

Description of the Agricultural Production Zones and Selected Enterprises in Uganda (MAAIF,2005).

Table A.1.1 Zones, districts and climatic as well as on-going development activities

Zone	Districts	Climate	Other characteristics
I	1. Moroto 2. Northern Kotido 3. Eastern Kitgum	• Average annual rainfall of 745 mm with high variability, from about 600 mm over the north and northeastern parts to about 1000 mm over the southern and western parts. • One rainy season of about 5½ months, from April to early September with the main peak in July/August and a secondary peak in May. One long dry season of about 6 months from October to March. Most dry months are between December to February. • Evaporation exceeds rainfall by a factor of over 10 during the driest months, December to February. During the rainy season evaporation is slightly more than rainfall. • Temperature ranges from12.5 – 32.5 °C • Altitude ranges from 351 – 1,524 m ASL	• Generally flat with isolated hills • Land still abundant in this zone • Shifting cultivation is practiced • Soils are moderate to poor • With largely subsistence farming and pastoral activities • No natural open water bodies • Area is semi-arid • With poorly underdeveloped infrastructure • Ongoing programmes include Northern Uganda Sustainable Agriculture Fund (NUSAF), Kotido Development Agency (KDA), and World Food Programme (WFP) • There is civil strife and cattle rustling
II	1. Pader 2. Kitgum 3. Eastern Lira 4. Katakwi 5. Northern Sironko 6. Northern Kapchorwa 7. Nakapiripirit 8. Southern Kotido	• Average annual rainfall of 1197 mm with moderate variability, from about 1000 mm over the north and north-eastern parts to about 1300 mm over western and southern parts • One rainy season of about 7 months, from April to late October with the main peak in July/August and a secondary peak in May. One long dry season of about 4 months from mid-November to late March. Driest months are from December to February. Evaporation exceeds rainfall by a factor of over 10 during the driest months, December to February. During the rainy months, May; July and August rainfall is slightly more than evaporation • Temperature ranges from 15 - 32.5 °C • Altitude ranges from 975 – 1,524 m ASL	• Generally flat with isolated hills • Land is available but mainly in communal ownership • Shifting cultivation is practiced • Soils are moderate to poor • Wide wetlands with potential for irrigation • Farming is mainly communal and predominantly subsistence with emerging commercial farms • There is communal labour for cultivation • There is moderate to high literacy • Moderate infrastructure development • Area suffers from low incidental strife • Programmes include Northern Uganda Sustainable Agriculture Fund (NUSAF), Olweny Rice Scheme, and Voluteer Organization for Displaced People (VODP)

Table A. 1.1 continued

Zone	Districts	Climate	Other characteristics
III	1. Adjumani 2. Western Nebbi 3. Arua 4. Moyo 5. Yumbe 6. Northern Gulu 7. Northern Apac 8. Western Lira	• Average annual rainfall range of 1340 mm – 1371mm • Moderate variability, from about 1200 over northwestern and western parts to about 1500 mm over the southern parts. • One rainy season, about 7½ from months, April to about mid November with the main peak in August to mid October and a secondary peak in April/May. One long dry season of about 4 months from mid-November to late March. Driest months are December to February. Evaporation exceeds rainfall by a factor of up to 10 during the driest months from December to February. During the rainy months of May, August and September rainfall exceeds evaporation. • Temperature ranges from 15 - 25 °C • Altitude ranges from 351 – 1,341 m ASL	• Generally flat with undulating hills • Good to moderate soils • Mainly small holder food and cash crop farming with subsistence in some areas • Numerous small perennial streams • Moderate literacy levels • Have largely poor incomes due to high levels of strife • Largely peaceful but with influx of refugees • Infrastructure is moderate to poor • There is a possibility of block farming on a large scale in the short term due to the Internally Displaced Peoples (IDPs) • Abundance of land in this zone, and hence there is high potential for increased production • Outgrower systems existing • Have advantage of cross border trade with DR Congo and Sudan • On going programmes are NUSAF. Rural Electrification Programme, NAADS, Northwest Smallholder Agric project
IV	1. Eastern Nebbi 2. South-western Gulu 3. Western Masindi	• Average annual rainfall of 1259 mm with high variability, from about 800 within the Lake Albert basin to about 1500 mm over the western parts • Mainly one rainy season of about 8 months, from late March to late November with the main peak from August to October and a secondary peak in April/May. One long dry season of about 3½ months, from December to about mid March. Driest months are December to February. Evaporation exceeds rainfall by a factor of about 6 during the driest months from December to March. During the rainy season, July to October, evaporation exceeds rainfall. • Temperature ranges from 17.5 – 32.5 °C • Altitude ranges from 351 – 1,341 m ABL	• Largely parkland with potential for livestock ranching. • Generally flat with undulating hills • Good to moderate soils • Moderate literacy levels • Largely peaceful but with influx of refugees • Infrastructure is moderate to poor • There is a possibility of block farming on a large scale in the short term due to the internally displaced camps • Land available in this zone and hence there is high potential for increased production • Have advantage of cross border trade with DR Congo • On going programmes are NUSAF. Northwest Smallholder Agric project, Uganda Wildlife Authority (UWA) programme

Table A.1.1 continued

Zone	Districts	Climate	Other characteristics
V	1. Kayunga 2. Kamuli 3. Iganga 4. Northern Bugiri 5. Tororo 6. Northern Busia 7. Southern Mbale 8. Pallisa 9. Kumi 10. Soroti 11. Kaberamaido 12. Southern Lira 13. Southern Apac	• Average annual rainfall range of 1215 mm - 1328 mm is recorded • Two rainy seasons in the southern part with the main season from March to May with peak in April, and secondary season from August to November with a peak in October/November. Main dry season December to February, secondary dry season is June and July. Evaporation exceeds rainfall by a factor of about 3mm during the dry months of December to February. During the main rainy season rainfall is greater and or about equal to evaporation. • Virtually one rainy season in the northern part from March to November, with the main peak in April/May and a secondary peak in August/September. One dry season December to about mid March. Evaporation exceeds rainfall by a factor of about 8mm during the dry months December to February. During the main rainy season rainfall is greater and or about equal to evaporation • Temperature ranges from 15 – 32.5 °C • Altitude ranges from 914 – 1,800 m ASL	• Land flat and swampy • Soils are poor to moderate • Small-scale subsistence mainly annual crops with some pastoralism • Some commercial farms • Moderate to low literacy levels • Fairly well endowed with resources • Stable geo-politically • Land is available for commercial farming • On going programmes include NAADS and many others
VI	1. Kampala 2. Mukono 3. Wakiso 4. Eastern Mpigi 5. Eastern Masaka 6. Eastern Rakai 7. Kalangala 8. Jinja 9. Mayuge 10. Southern Bugiri 11. Southern Busia	• Average annual rainfall of 1,200 to 1,450 mm • Two rainy seasons in the eastern part of the zone with the main season from March to May with peak in April and secondary season from August to November with a modest peak in October/November. • Main dry season December to February, secondary dry season is June to September. • Evaporation exceeds rainfall by a factor of about 2mm during the dry months, December to February. During the peak of the rainy seasons rainfall is greater and or equal to evaporation • Two rainy seasons in the western part of the zone with the main season March to May with peak in April and secondary season October to December with a peak in November. Main dry season for June to September, secondary dry season is January and February. Evaporation exceeds rainfall by a factor of about 3mm during the dry months, June to August. During the main rainy season rainfall is greater and or equal to evaporation. • Temperature ranges from 15 – 30 °C • Altitude ranges from 1,000 – 1,800 m ABL	• Hilly and flat with wetland and forested areas • Soils are good to moderate • Small medium and large-scale intensive farming with potential for commercial production • Infrastructure generally good. Prospects for processing zones and warehousing • Entrepreneurship skills are fairly well developed. • Skilled labour is readily available • Service providers available • Attitudes positive and open to new technology • Literacy levels fairly high • Generally well endowed with resources • Stable and mostly cosmopolitan with high potential for peri-urban farming • Land is generally available through sale • Lots of private investment opportunities • High potential for irrigation • Higher migrant labour available • Water for industry available • Most on going programmes are present here

Table A.1.1 continued

Zone	Districts	Climate	Other characteristics
VII	1. Hoima 2. Kiboga 3. Southern Luwero 4. Mubende 5. Kibaale 6. Kyenjojo 7. Kabarole 8. Kamwenge 9. Southern Kasese	• Average annual rainfall of 1,270 mm with high variability, from about 800mm over eastern L. Albert parts to about 1400mm over the western parts. • Two rainy seasons, main season from August to November with peak in October and secondary season March to May with peak in April. The main dry season is from December to about mid March; secondary dry season is June to July. Evaporation exceeds rainfall by a factor of up to 5mm during the dry months. During the rainy months, rainfall is greater or equal to evaporation. • Temperature ranges from 15 – 30 °C • Altitude ranges from 621 – 1,585 m ASL	• Soils are generally good to moderate • Land available for agriculture, but under utilized • Small to large scale farming, but majority are smallholder • Outgrower systems exist • Infrastructure moderately developed • There are land ownership disputes in some parts • On going programmes include, National Agricultural Advisory and Development Services (NAADS), District Decentralized Services Programme (DDSP), and Fisheries Development Project (FDP)
VIII	1. Eastern Masindi 2. Nakasongola 3. Northern Luwero 4. Central Kiboga 5. Southern Mubende 6. Western Mpigi 7. Western Masaka 8. Western Rakai 9. Sembabule 10. Eastern Mbarara 11. Southern Ntungamo 12. Northern Bundibugyo	• Average annual rainfall range of 915 to 1021 mm • Two rainy seasons, main season from March to May with peak in April and secondary season in September to December with a modest peak in November. Main dry season June to August, secondary dry season is January to February. Evaporation exceeds rainfall by a factor of about 6 during the dry months in June to August. During the main rainy months, April and May rainfall equals evaporation • Temperature ranges from 12.5 – 30°C • Altitude ranges from 129 – 1,524 m ASL	• Rolling hills with some flat areas • Soils are moderate to poor • Mainly small holders with a lot of communal grazing • Agro pastoral practices • Low literacy level • Absentee landlords with squatter population • Infrastructure and marketing systems are poor to moderate • National Livestock Productivity Improvement Project (NLPIP) is an on going programme
IX	1. Western Mbarara 2. Bushenyi 3. Northern Ntungamo 4. Rukungiri 5. Northern Kanungu	• Average annual rainfall range of 1,120 – 1,223 mm • High variability, lowest about 800 mm in Kasese Rift Valley, highest over slopes of Rwenzori mountains, over 1500mm • Two rainy seasons, main season from August to November with peak in September to November and secondary season in March to May, with peak in April. For Mubende and Luwero the main season is March to May with a peak in April and the Secondary season from October to December with a peak in November. • Evaporation exceeds rainfall by a factor of about 5 during the dry months from December to March. During the rainy months of March, and August to November rainfall exceeds evaporation. Main dry season is from December to late March, secondary dry season is June to August. • Temperature ranges from 12.5 – 30°C • Altitude ranges from 129 – 1,524 m ASL	• Shortage of land and land fragmentation in some parts of the zone • Largely small to medium scale intensive farming • Potential for block farming e.g. in Kasese • Moderate literacy rate • Relatively well organised and moderately endowed • Infrastructure and marketing systems are fairly well developed • Farmers' entrepreneurial skills are well developed • Attitudes towards farming are good • Ongoing programmes include NAADS, Area-based Agricultural Programme (AAP), International Fund for Agricultural Development (IFAD), and FDP

Table A.1.1 continued

Zone	Districts	Climate	Other characteristics
X	1. Northern Mbale 2. Southern Sironko 3. Southern Kapchorwa 4. Southern Kanungu 5. Kabale 6. Kisoro 7. Northern Kasese 8. Southern Bundibugyo	• Average annual rainfall usually of more than 1400 mm • Two main rainy seasons, i.e., from September to December for the Kabale, Kisoro and Kasese region • One long rainy season from March to October with peak in April and Secondary peak in August for Northern Mbale, Southern Sironko, Southern Kapchorwa • Temperature ranges from 7.5 – 27.5 °C • Altitude ranges from 1,299 – 3,962 m ASL	• Soils are mostly young volcanic and are rich in nutrients • Mountainous high altitude areas • Cultivated land is highly fragmented with small plots covering terraced hillsides • Infrastructure is poor largely due to the terrain • Entrepreneurial skills fairly developed • Stable geo-politically • On going programmes include NAADS, Agro Forestry, African Highlands initiatives, AFRICARE, IUCN, CARE

Annex 2:

Crops Found in Different Agro-ecological Zones Described Above

Table A.2.1: Scientific names of some characteristic crops found in agro-ecological zones of Uganda (NARO, 1999)

Type	***Common name***	***Scientific name***
Cereals		
	Sorghum	*Sorghum bicolor*
	Finger millet	*Eleusine coracana*
	Pearl millet	*Pennisetum americanum*
	Rice	*Oryza sativa*
	Maize	*Zea mays*
	Wheat	*Triticum aestivum*
Legumes		
	Beans	*Phaseolus vulgaris*
	Groundnut	*Arachis hypogaea*
	Pigeon peas	*Cajanus cajan*
	Cowpeas	*Vigna unguiculata*
Oil crops		
	Sesame	*Sesamum indicum*
	Sunflower	*Helianthus annuus*
Spices		
	Onions	*Allium cepa, A.sativum*
	Vanilla	*Vanilla fragrans*
Fruits		
	Citrus	*Citrus* spp.
	Passion fruits	*Passiflora* spp.
	Banana	*Musa* spp.
Bevarages		
	Coffee (Robusta and Arabica)	*Coffea canephora, and C.arabica*
	Tea	*Camellia sinensis*
Roots and Tubers		
	Cassava	*Manihot esculenta*
	Sweet potato	*Ipomea batatas*
	Irish potato	*Solanum tuberosum*
Fiber crops		
	Cotton	*Gossypium hirsutum*

Annex 3:

Preliminary Observations Made in Relation to The Survey Report in Chapter 3

3.1 Farmer field practices observed during the survey and extent to which they are used

Agronomic practices used by the farmers surveyed were: rotation, intercropping, staking, pruning, mulching, uprooting and field irrigation. The extent to which these methods were used is indicated in Table A.3.1.

The practice of tomato seed extraction by farmers themselves was very common with over 57% of interviewed farmers extracting their seed irrespective of whether the variety was indigenous or improved. In Rakai district, all interviewed farmers extracted their own seed. Mbarara district came second with 80% farmers extracting their own seed. Other districts had less than 50% farmers extracting their own seed, for example Mbale and Mukono with 20% each, used lowest amounts of farmer-extracted seed. Farmers extracted their own seed because there was irregular supply of certified seed to village markets and market prices were often prohibitive.

3.2-Tomato varieties and other crops grown by farmers

It was established that 28.6% of all farmers visited, used the indigenous varieties, Kifudu, and Nganda, while the rest depended on exotic varieties, such as Heinz, Mangrobe, Money Maker, and Roma. In general crops grown in the agro-ecosystem included maize, beans, cassava, sesame, sugar cane, onions, curcubits, passionfruits, banana, sweet potato, coffee, cabbage, solanum potato, cotton, and sweet paper (Table A.3.2).

3.3-Pesticide application against insect pests

In 1996-97 preliminary survey, insect pests/vectors observed on visited farmers fields in districts of Iganga, Kasese, Mbale, Mpigi, Mbarara, Mukono, and Rakai were thrips (*Thrips tabaci*), green aphids (*Myzus persicae*), whiteflies (*Bemisia tabaci*), and

leafhoppers (*Circulifer tenellus*). Thrips were most numerous, and most common, especially where respondent farmers used insecticides (Table A.3.3 and A.3.4).

Considering vector occurrence, whiteflies were found mostly on young vegetatively vigorous tomato plants; whereas white thrips, aphids and leafhoppers were observed at all tomato growth stages. In Mpigi district, aphid populations were not observed in the fields visited whereas whiteflies and thrips were found in all fields. In contrast, in Mbarara district whiteflies occurred in only 20% of fields visited, whereas no aphids or leafhoppers were observed in the field. Thrips were, however, found in every field visited. In Kasese district all four types of insect pests occurred, i.e. whiteflies, aphids, thrips and leafhopper. Pesticides were used on tomato in all districts with their lowest use being in Mukono and Rakai districts, where 75% and 60% of farmers applied pesticides, respectively. A wide range of pesticides was used. These were Rogor (Dimethoate), Dursban, Fenom P. Ambush (Pyrethroid), Dimecron (Phosphamidon), and Sumithion (Fenitrothion) (Table A.3.4).

Table A.3.1: Percentage number of respondents (10 or more respondents per district) applying various agronomic practices per district surveyed. Agronomic practices included use of indigenous (traditional) varieties, seed extraction, rotation, intercropping, irrigation, staking, pruning, uprooting, and mulching

District	*Indigenous variety*	*Farmer seed extraction*	*Rotation*	*Intercropping*	*Irrigation*	*Staking*	*Prunning*	*Uprooting*	*Mulching*
Rakai	100	100	100	80	0	0	20	20	80
Mbale	0	20	100	20	0	100	100	0	0
Iganga	0	40	100	20	0	0	40	0	80
Mpigi	80	100	100	60	0	0	60	0	60
Mukono	20	20	80	0	0	0	20	0	40
Mbarar	0	80	80	20	0	0	80	0	0
Kasese	0	40	100	20	60	80	80	0	0
Mean	29	57	94	31	9	26	57	3	37

Table A.3.2: Crops associated with tomato fields in Iganga (IG) Kasese (KA), Mbale (MB), Mpigi (MP), Mbarara (MR), Mukono (MU), and Rakai (RA) districts as either intercrops or rotation crops. Crops observed in the district were recorded as (+), and those not observed as (-)

Crops	*IG*	*KA*	*MB*	*MP*	*MR*	*MU*	*RA*
Maize	+	+	+	+	+	+	+
Beans	+	+	+	+	+	+	+
Cassava	+	+	+	+	+	+	+
Sesame	-	-	-	+	-	-	-
Sugarcane	-	-	-	+	-	-	-
Onions	-	+	-	-	-	+	-
Curcubits	-	+	-	-	-	-	-
Passionfruits	-	+	-	-	-	-	-
Banana	+	+	+	-	+	-	+
Sweet potato	+	-	-	-	-	-	+
Coffee	+	-	-	-	-	-	-
Cabbage	-	-	-	-	+	+	-
Solanum potato	-	-	+	-	-	-	-
Cotton	-	-	-	+	-	+	-
Sweet pepper	-	-	+	-	-	+	-

Table A.3.3: Percentage presence of insect pests in tomato fields visited per district, in Uganda

Districts	*Observed percentage number of fields with various insect pests per district (%)*			
	Whiteflies	Aphids	Thrips	Leafhoppers
Iganga	40	20	80	0
Kasese	80	80	100	60
Mbale	100	60	100	0
Mpigi	100	0	100	0
Mbarara	20	0	100	0
Mukono	100	25	100	25
Rakai	80	20	80	0
Mean	74	29	94	12

Table A.3.4: Percentage use of particular pesticides on tomato fields per district

Pesticide	*Iganga*	*Kasese*	*Mbale*	*Mpigi*	*Mbarara*	*Mukono*	*Rakai*	*Mean*
Ambush	0	0	100	80	0	25	20	32
Dimecron	0	0	0	20	0	0	0	3
Rogor	80	0	0	0	40	0	0	17
Dursban	0	0	6	0	0	50	0	8
FenomP	0	80	0	0	0	50	0	19
Sumithion	80	0	0	0	20	0	0	14
Others	0	20	0	20	0	25	40	15
Mean	23	14	15	17	9	21	9	

Most farmers sprayed with Ambush, which was followed by Fenom P. Dimecron was the least used insecticide. On average 15% of the farmers applied non-chemical pest management options, such as chili, ash and various botanical concoctions.

3.3-Weeds observed in tomato-growing ecosystems for each district surveyed

Fields were infested with a multitude of weeds as shown in table A.3.5 below.

Table A.3.5: Weeds observed in and around tomato fields surveyed

Weeds	*IG*	*KA*	*MB*	*MP*	*MR*	*MU*	*RA*
Solanum nigrum	-	-	-	-	-	+	-
Commelina benghalensis	+	+	-	+	+	+	+
Tagetes minuta	-	-	-	-	+	-	-
Bidens pilosa	+	+	+	+	+	+	+
Amaranthus dubius	-	-	+	-	+	-	-
Galinsoga parviflora	+	+	+	+	+	+	+
Desmodium spp.	-	-	-	-	-	+	-
Imperata cylindrica	+	-	-	-	+	-	-
Oxalis latifolia	+	+	+	+	-	-	-
Zinnia spp.	+	-	-	-	-	-	-
Euphorbia heterophyllum	+	-	-	-	-	-	-
Datura stramonium	-	+	-	-	-	-	-
Physalis floridana	+	-	-	-	-	-	+
Pennisetum spp.	-	+	-	-	-	+	-
Digitaria scalarum	-	+	-	+	-	-	+
Nicotiana tabacum	-	-	-	-	-	-	+
Panicum spp.	+	-	-	-	-	-	+
Cylinga spp.	-	-	-	-	-	-	+

(+) = weeds present in a district and (-) = weeds not observed during this preliminary survey

3.6-Relationship between farmer practices observed during our preliminary survey

There was a positive relationship between virus incidence and farmer extraction of seeds, rotation, pruning, irrigation, intercropping, staking, and aphid infestation, as shown in table A. 3.6 below.

There was a closer relationship between virus incidence and symptom expression than between agronomic practices. Virus incidence was negatively related to use of indigenous varieties, uprooting, mulching, use of pesticides and whitefly pest occurrence. There was a positive relationship between pesticides use and incidence of whiteflies (R = 0.074), aphids (R = 0.074) and leafhoppers (R = 0.104), which indicated that pesticide application enhances pest population or presence of pests induces farmer application of pesticides, but there was a negative relationship (R = -0.037) with thrips infestation and incidence of viral symptoms (R = -0.159).

See table A.3.6 below, whereby: (1) viral symptom incidence; (2) indigenous varieties; (3) self seed extraction; (4) rotation; (5) pruning; (6) uprooting; (7) mulching; (8) irrigation; (9) Inter-cropping; (10) staking; (11) pesticide use; (12) whitefly infestation; (13) aphid infestation; (14) Thrips infestation; (15) leafhopper infestation; and (16) represent viral symptom expression.

Table A.3.6: Correlation matrices (a-d)

	2	**3**	**4**	**5**	**1**
2	1.0				
3	0.8	1.0			
4	0.3	0.1	1.0		
5	-0.5	-0.2	0.2	1.0	
1	-0.03	0.5	0.03	0.5	1.0

a) Matrix determinant = 0.18

	6	**7**	**8**	**9**	**1**
6	1.0				
7	0.5	1.0			
8	-0.2	-0.4	1.0		
9	0.8	0.6	-0.2	1.0	
1	-0.2	-0.4	0.6	0.2	1.0

b) Matrix determinant = 0.23

	10	**11**	**12**	**13**	**1**
10	1.0				
11	-0.1	1.0			
12	0.4	0.5	1.0		
13	0.4	0.1	0.1	1.0	
1	0.1	-0.5	-0.3	0.3	1.0

c) Matrix determinant = 0.46

	14	**15**	**16**	**1**
14	1.0			
15	0.4	1.0		
16	-0.5	0.5	1.0	
1	0.3	0.4	0.1	1.0

d) Matrix determinant = 0.17

Annex 4:

Viruses Infecting Tomato Worldwide

Without any reference to virus genera, all virus names in italics have been accepted by ICTV, while those in normal font are yet to be accepted (http://image.fs.uidaho.edu/vide/descr)

- Alfamoviruses

Alfalfa mosaic alfamovirus

- Bigeminiviruses

Abutilon mosaic bigeminivirus, Chino del tomat bigeminivirus, Croton yellow vein mosaic bigeminivirus, Okra leaf-curl bigeminivirus,Pepper mild tigr (?) bigeminivirus, Pepper Texas bigeminivirus, Potato yellow mosaic bigeminivirus, Serrano golden mosaic bigeminivirus, Solanum apical leaf curling (?) bigeminivirus, Soybean crinkle leaf (?) bigeminivirus, Tobacco leaf curl bigeminivirus, Tomato mottle bigeminivirus, Tomato Australian leafcurl bigeminivirus, Tomato golden mosaic bigeminivirus, Tomato yellow leaf curl bigeminivirus, Tomato yellow mosaic bigeminivirus

- Carlaviruses

Cassia mild mosaic (?) carlavirus, Pea streak carlavirus, Cowpea mild mottle (?) carlavirus, and Potato M carlavirus

- Carmoviruses

Carnation mottle carmovirus, Elderberry latent (?) carmovirus, Turnip crinkle carmovirus, and Pelargonium line pattern (?) carmovirus

- Closteroviruses

Tomato infectious chlorosis (?) closterovirus

- Comoviruses

Potato Andean mottle comovirus

- Cucumoviruses

Cucumber mosaic cucumovirus, Peanut stunt cucumovirus, and Tomato aspermy cucumovirus

- Cytorhabdoviruses

Lettuce necrotic yellows cytorhabdovirus

- Dianthoviruses

Red clover necrotic mosaic dianthovirus

- Furoviruses

Potato mop-top furovirus, Peanut clump furovirus

- Hybrigeminiviruses

Beet curly top hybrigeminivirus, and Tomato pseudo curly top (?) hybrigeminivirus

- Ilarviruses

Elm mottle ilarvirus, Tobacco streak ilarvirus, Parietaria mottle ilarvirus, and Sunflower ringspot (?) ilarvirus

- Ipomoviruses

Sweet potato mild mottle ipomovirus

- Latentviruses

Wineberry latent virus

- Luteoviruses

Beet western yellows luteovirus, Potato leafroll luteovirus, Tobacco vein-distorting (?) luteovirus, Tobacco yellow net (?) luteovirus, Tobacco necrotic dwarf luteovirus, Tobacco yellow vein assistor (?) luteovirus

- Monogeminiviruses

Chickpea chlorotic dwarf (?) monogeminivirus, Tobacco yellow dwarf monogeminivirus

- Necroviruses

Tobacco necrosis necrovirus

- Nepoviruses

Arabis mosaic nepovirus, Arracacha A nepovirus, Arracacha B (?) nepovirus, Blueberry leaf mottle nepovirus, Tomato black ring nepovirus, Tomato ringspot nepovirus, Tomato top necrosis (?) nepovirus, Potato black ringspot nepovirus, Cassava green mottle nepovirus, Potato U nepovirus,, Raspberry ringspot nepovirus, Rubus Chinese seed-borne (?) nepovirus, Strawberry latent ringspot (?) nepovirus, Dogwood mosaic (?) nepovirus, Tobacco ringspot nepovirus

- Nucleorhabdoviruses

Tomato vein clearing nucleorhabdovirus, Datura yellow vein nucleorhabdovirus, Eggplant mottled dwarf nucleorhabdovirus, Pittosporum vein yellowing nucleorhabdovirus

- Ourmiaviruses

Epirus cherry ourmiavirus, Melon Ourmia ourmiavirus, Pelargonium zonate spot ourmiavirus

- Phytoreoviruses

Clover wound tumor phytoreovirus

- Potexviruses

Foxtail mosaic potexvirus, Commelina X potexvirus, Pepino mosaic potexvirus, Nerine X potexvirus, Plantain X potexvirus, Potato aucuba mosaic potexvirus, Tamus latent (?) potexvirus, Tulip X potexvirus, White clover mosaic potexvirus

- Potyviruses

Datura distortion mosaic potyvirus, Datura innoxia Hungarian mosaic (?) potyvirus, Datura mosaic (?) potyvirus, Datura necrosis potyvirus, Eggplant green mosaic potyvirus, Eggplant severe mottle (?) potyvirus , Groundnut eyespot potyvirus, Henbane mosaic potyvirus, Pepper Indian mottle potyvirus, Pepper mottle potyvirus, Plum pox potyvirus, Potato V potyvirus, Potato Y potyvirus, Tamarillo mosaic potyvirus, Tobacco etch potyvirus, Tomato mild mottle (?) potyvirus, Tulip chlorotic blotch potyvirus, and Carrot mosaic (?) potyvirus, Brinjal mild mosaic (?) potyvirus, Marigold mottle potyvirus, Melilotus mosaic (?) potyvirus, Pepper veinal mottle potyvirus, Pepper severe mosaic potyvirus, Tobacco vein mottling potyvirus, Wild potato mosaic potyvirus, Soybean mild mosaic virus, Tomato Peru potyvirus

- Satelliviruses

Tobacco mosaic satellivirus

- Sequiviruses

Parsnip yellow fleck sequivirus

- Tobamoviruses

Maracuja mosaic (?) tobamovirus, Potato 14R (?) tobamovirus, Ribgrass mosaic tobamovirus, Rose (?) tobamovirus, Tobacco mild green mosaic tobamovirus, Tobacco mosaic tobamovirus, Tomato mosaic tobamovirus, Ullucus mild mottle tobamovirus

- Tobraviruses

Tobacco rattle tobravirus, Pepper ringspot tobravirus

- Tombusviruses

Cymbidium ringspot tombusvirus Pepper Moroccan tombusvirus, and Tomato bushy stunt tombusvirus,

- Tospoviruses

Tomato chlorotic spot (?) tospovirus, and Tomato spotted wilt tospovirus.

- Tymoviruses

Abelia latent tymovirus, Physalis mosaic tymovirus, Potato Andean latent tymovirus,Ononis yellow mosaic tymovirus, Dulcamara mottle tymovirus,and Eggplant mosaic tymovirus.

- Umbraviruses

Tobacco yellow vein (?) umbravirus, and Tobacco mottle umbravirus

- Varicosaviruses

Tobacco stunt varicosavirus

Annex 5:

Table A.5.1: Detailed results of DNA Hybridisation using both specific and general probes

Locarion on X-ray film membrane	Sample code	General probe	Specific probe TYLCV	Sample Code	Specfic probe Overnight	Specific probe 2 days
1A	MB1 28	-[1]	-	IG1	++	+++
1B	MB2 28	-	-	IG2	-	-
1C	MB3 28	-	-	RL5	+[2]	+++
1D	MB4 28	-	-	K2	-	-
1E	ISO 2	-	-	K3	+	+++
1F	ISO 3	-	-	-ve Control	-	-
1G	ISO 4	-	-			
1H	ISO 5	-	-			
1I	ISO 6	-	-			
2A	ISO 6	-	-	CVMV	+	++
2B	ISO 7	-	-	K1	++	+++
2C	ISO 8	-	-	RL1	+	++
2D	ISO 9	-	-	RL2	+	+++
2E	ISO 10	-	-	ISOPOT	+	+++
2F	ISO 11	-	-	+ve Control	+++	+++
2G	ISO 12	-	-			
2H	ISO 13	-	-			
2I	ISO 14	-	-			
3A	ISO 15	-		MB8	[3]0	++
3B	ISO 16	-	-	CVMVmx	0	++
3C	ISO 17	-	-	MB6	0	
3D	ISO 18	-	-	MB9	+	++
3E	ISO 19	-	-	MB7	0	-
3F	ISO 20	-	-			
3G	ISO 21	-	-			
3H	ISO 22	-	-			
3I	ISO 23	-	-			
4A	ISO 24	-	-			
4B	ISO 25	-	-			
4C	ISO 26	-	-			
4D	ISO 27	-	-			
4E	ISO 28	-	-			

[1] negative reaction, [2] positive reaction, and [3](0) a blank means that the sample was not tested

Table A.5.1 continued

Locarion on X-ray film membrane	Sample code	Genera l probe	Specific probe TYLCV	Sample Code	Specfic probe Over night	Specific probe 2 days
4F	ISO 29	-[1]	-			
4G	ISO 30	+++	+[2]	[3]		
4H	ISO 31	-	-			
5A	IG10/22	+++	-			
5B	IG7/ 22	-	+			
5C	MB4 22	-	-			
5D	MB20/22	-	+			
5E	MB2 22	-	-			
5F	MB5 22	-	-			
5G	MB3 22	-	-			
5H	MB10/22	-	-			
5I	MB21/22	-	+			
6A	RL 5	-	-			
6B	IG1 3	++	-			
6C	IG2/3	-	+			
6D	IG2/3	-	+			
6E	IG2/3	-	+			
6F	K2/3	-	-			
6G	TYLCV	+++	+++			
6H	BGMV	++	-			
6I	-ve	-	-			

[1] negative reaction, [2] positive reaction, and [3] a blank means that the sample was not tested

Annex 6:

Figure A.6.1: CLUSTAL W (1.82) nucleotide multiple sequence alignment of (a) 521bp of coat protein region, (IR, V1, V2 ORFs), and (b) 482bp of replicative gene (C1 ORF) for tomato leaf curl virus (ToLCV) from Uganda with geminiviruses OkLCV-EG, CoLCV-SD, ToLCV-Ma, TbLCZWV, TbLCZWmd, ATLCV-Tz, EACMV-UG, AEV-NP, TYLCV-Is, TYLCV-JR, TYLCV-OM, TLCV-SD, ToLCV-IN, ToLCV-TW, TbLCV-Ph, SCLV-JR, ToLCV-PH, TCSV-SA, ToLCV-AU and ChaMV-Ng for outgroup rooting

(-) indicates no consensus, while areas of maximum consensus are marked with asterisks

Fig.A.6.1a) ToLCV-Ugrev

```
TbLCZbwV         --------------------------------TATTGGGCCTCCAA--GGCCGCGCAGCGG 27
TbLCZbwV-md      --------------------------------TAAACGCTTTTGTATTAGCCGCGCAGCGG 29
ATLCV-Tz         --------------------------------TCATGGGTCTTAAT--GGCCGCGCAGCGG 27
ChaMV-Ng         --------------------------------TACTCGGCCTCCAC--GGCCGCGCAGCGG 27
AEV-NP           --------------------------------TTTGAGGCCTCAGA--GGCCGCGCAGCGG 27
OkLCV-EG         --------------------------------TAATCGGTCTACTT--GGCCGCGCAGCGG 27
CoLCV-SD         --------------------------------TAATCGGTCTACTT--GGCCGCGCAGCGG 27
ToLCV-Ma         --------------------------------TATTGGGTCTCCAA--GGCCGCGCAGCGG 27
ToLCV-UGrev      --------------------------------TGTGGGATCCACTGTTAA---ATGAGTTC 26
EACMV-MW         --------------------------------TGTGGGATCCATTGTTGA---ATGAGTTC 26
TYLCV-Is/PT      --------------------------------TGTGGGATCCACTTCTAA---ATGAATTT 26
TYLCV-JR/Szka    --------------------------------TGTGGGATCCACTTCTAA---ATGAATTT 26
TYLCV-OM         --------------------------------TGTGGGACCCACTTCTAA---ATGAATTT 26
TLCV-SD/Gez      --------------------------------TGTGGGATCCACTTCTAA---ATGAATTT 26
ToLCV-IN/Bg      --------------------------------TGTGGGATCCACTTACAA---ATGATTTT 26
ToLCV-TW         --------------------------------TGTGGGATCCACTTTTAA---ATGATTTT 26
                                                 *      *               *

TbLCZbwV         CACCACACACATTCTCA---GAGACCCACCCTTCAAGTTCCTCAGGGACTTGATCAAAAG 84
TbLCZbwV-md      CACCACACACATTCTCA---GAGACCCACTCTTCAAGTCCCTCAGGGACTTGATCAAAAG 86
ATLCV-Tz         TAGTTAGAACATTCTGT---GCCGCCCACTCTTCAAGTTCCTCTGGAACTTGATCGAAAG 84
ChaMV-Ng         CATCCATCACGTTCGCC---GATGCCCATCGTTGAAGTTCATCAGGAACCTGAGTGAAGG 84
AEV-NP           CATTCATGACATTTTCT---GAGACCCATTCTTCAAGTTCTTCAGGAACTTGATCAAAAG 84
OkLCV-EG         CCTCAACGACGTTCTCC---GCCGCCCATTCTTCAAGTTCTTCGGGAACTTGGTCGAACG 84
CoLCV-SD         CATCAACGACGTTCTCC---GCCGCCCATTCTTCAAGTTCTTCTGGAACTTGGTCGAACG 84
ToLCV-Ma         CATCCCTGACGTTCTTA---GAAACCCACTCTTCAAGTTCTTCGGGAACTTGATCGAAAG 84
TbLCV-Ch         CATGCCTTACATTATCAA--GCAGCCCACTCTTCAAGTTCTTCGGGAACTTGATCAAATG 85
ToLCV-UGrev      CCAGACTC-TGTTC------ATGGGT-TTCG---TTGCAT-----GCTTGCTATAAAATA 70
EACMV-MW         CCCGATTC-TGTGC------ACGGTT-TTCG---CTGTAT-----GCTTGCTATTAAATA 70
TYLCV-Is/PT      CCTGAATC-TGTTC------ACGGAT-TTCG---TTGTAT-----GTTAGCTATTAAATA 70
TYLCV-JR/Szka    CCTGAATC-TGTTC------ACGGAT-TTCG---TTGTAT-----GTTAGCTATTAAATA 70
TYLCV-OM         CCTGAATC-TGTTC------ACGGAT-TTCG---TTGTAT-----GTTAGCTATTAAATA 70
TLCV-SD/Gez      CCTGAATC-TGTTC------ATGGAT-TTCG---TTGTAT-----GTTAGCTATTAAATA 70
ToLCV-IN/Bg      CCCGAAAC-CGTAC------ACGGAT-TCAG---GTGTAT-----GCTTGCTATAAAATA 70
ToLCV-TW         CCGGAGTC-TGTG--C----ATGGTC-TACG---GTGTAT-----GCTTGCCATTAAGTA 70
                           *                                  *         *

TbLCZbwV         A--AGAAGAAGAGAAA-GGAGAAATATAAGGCTCTGGGGGAGCCTGGAAAATC------- 134
TbLCZbwV-md      A--AGAAGAAGAGAAA-GGAGAAATATAAGGCTCTGGGGGAGCCTGGAAAATC------- 136
ATLCV-Tz         A--AGAACATAAGAAA-GGAGAAACATAATCCTCCAACGGAGGTGTAAAAATC------- 134
ChaMV-Ng         A--GGAAGCACAGAAG-GGAGAAACATAAACCTCCGGAGGAGCTTGGAAGATC------- 134
AEV-NP           A--AGAAGATAAAAAA-GGCGAAACATAAACCTCCAACGGAGGAGTAAAAATC------- 134
OkLCV-EG         A--AGAAGACAAAAAA-GGAGAAACATAAGGAGCTGGAGGCTCCTGAAAAATC------- 134
CoLCV-SD         A--AGAAGACAAAAAA-GGAGAAACATAAGGAGCTGGAGGCTCCTGAAAAATC------- 134
```

Fig.A.6.1a) continued

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ToLCV-Ma          A--AGAAGAAAGAAAA-GGACAAACAAAAACCTCTACAGGAGGTGCAAAAATC------- 134
TbLCV-Ch          A--TGAAGATAAAAAA-GGAGAAACAAAA-CCTCTAAAGGAGGTGCAAAAATC------- 134
ToLCV-UGrev       TTTGCAGGCTATTGAG-TCCA---CTTACGAGCC-CAATACTTTGGGCC--ACGATTTAA 123
EACMV-MW          TTTGCAGGCCTTGGAG-GAAA---CCTACGAGCC-CAATACTTTGGGCC--ACGATCTAG 123
TYLCV-Is/PT       TTTGCAGTCCGTTGAG-GAAA---CTTACGAGCC-CAATACATTGGGCC--ACGATTTAA 123
TYLCV-JR/Szka     TTTGCAGTCCGTTGAG-GAAA---CTTACGAGCC-CAATACATTGGGCC--ACGATTTAA 123
TYLCV-OM          TTTGCAGTCCGTTGAG-GAAA---CTTACGAGCC-CAATACATTGGGCC--ACGATTTAA 123
TLCV-SD/Gez       TTTGCAGGCCGTTGAG-GAAA---CTTACGAGCC-CAATACATTGGGCC--ACGATTTAA 123
ToLCV-IN/Bg       CTTGCAGTTGGTAGAA-AATA---CGTATTCCCC-CGATTCTTTGGGAT--ACGATCTAA 123
ToLCV-TW          TCTACATTTTGTAGAA-AATA---CATATTCCCCGGAT-ACTTTGGGTT--ACGATCTCA 123
                       *       *                                    ***** *
TbLCZbwV          ----------------------------------------------------CTA--TCTAAA 143
TbLCZbwV-md       ----------------------------------------------------CTA--TCTAAA 145
ATLCV-Tz          ----------------------------------------------------CTA--TCTAAA 143
ChaMV-Ng          ----------------------------------------------------TTA--TCCAAA 143
AEV-NP            ----------------------------------------------------CTA--TCTAAA 143
OkLCV-EG          ----------------------------------------------------CTC--TCTAAA 143
CoLCV-SD          ----------------------------------------------------CTC--TCTAAA 143
ToLCV-Ma          ----------------------------------------------------CTA--TCTAAA 143
TbLCV-Ch          ----------------------------------------------------CTA--TCTAAA 143
ToLCV-UGrev       TTCGA-------GAT-----------------------------------CTCA--TTTGTG 141
EACMV-MW          TCCGT-------GAT-----------------------------------CTCA--TCTGTG 141
TYLCV-Is/PT       TTAGG-------GAT-----------------------------------CTTA--TATCTG 141
TYLCV-JR/Szka     TTAGG-------GAT-----------------------------------CTTA--TATCTG 141
TYLCV-OM          TTAGG-------GAT-----------------------------------CTTA--TATCTG 141
TLCV-SD/Gez       TTAGG-------GAT-----------------------------------CTTA--TATCTG 141
ToLCV-IN/Bg       TACGT-------GAT-----------------------------------TTAA--TTTCTG 141
ToLCV-TW          TACGT-------GAT-----------------------------------TTGA--TTTTAG 141
                                                                      *  *
TbLCZbwV          TTAGT-----CTTTAAATTATGATACTGAAAAATAAAATCTTTAGGGAGTTTTTCCCTAA 198
TbLCZbwV-md       TTAGT-----CTTTAAATTATGATACTGAAAAATAAAATCTTTAGGGAGTTTTTCCCTAA 200
ATLCV-Tz          TTACA-----TTTTAAATTATGATACTGAAAAATGAAATCTTTAGGGAGTTTCTCCCTAA 198
ChaMV-Ng          TTAGC-----CTTGATATTATGATATTGAAATATATAATCTTTGGGGAGTTTCTCCTTAA 198
AEV-NP            TTAGA-----ATTTAAATTATGATATTGAAAAATAAATTTTTCTGGGAGTTTCTCCTTTA 198
OkLCV-EG          TTACT-----ATTTAAATTATGAAATTGTAAAACAAAATCTTTGGGAGCTAACTCCCTTA 198
CoLCV-SD          TTACT-----ATTTAAATTATGAAATTGTAAAACAAAATCTTTGGGAGCTAACTCCCTTA 198
ToLCV-Ma          TTAGA-----ATTTAAATTATGAAATTGTAAAACAAAATCTTTAGGAGCTAACTCCTTAA 198
TbLCV-Ch          TTAGC-----ATTTAAATTATGAAATTGTAAAACAAAATCTTTAGGAGCTAACTCCTTAA 198
ToLCV-UGrev       TCGTT---AGAGCCAGAGATTATGTCGAAGCGACCCGG---AGATATAATAATTTCAACG 195
EACMV-MW          TTATC---CGAGCCCGTGATTATGTCGAAGCGACCCGC---AGATATAATCATTTCCACT 195
TYLCV-Is/PT       TTGTA---AGGGCCCGTGACTATGTCGAAGCGACCAGG---CGATATAATCATTTCCACG 195
TYLCV-JR/Szka     TTGTA---AGGGCCCGTGACTATGTCGAAGCGACCAGG---CGATATAATCATTTCCACG 195
TYLCV-OM          TTGTA---AGGGCCCGTGACTATGTCGAAGCGACCAGG---CGATATAATCATTTCCACG 195
TLCV-SD/Gez       TTGTA---AGGGCACGTGACTATGTCGAAGCGACCAGG---CGATATAATCATTTCCACG 195
ToLCV-IN/Bg       TCGTC---AGGGC-----------------------------------TAAA---------- 155
ToLCV-TW          TTATC---CGTGCTAGAGATTATGTCGAAGCGACCCGC---CGATATAGTC--------- 186
                  *
TbLCZbwV          TAATAGCCATAGCTGCTTCAGCGGAACCTGCGTTTAAAGCGTCGGCGCATGCGTCGTTAG 258
TbLCZbwV-md       TAATAGCCATAGCTGCTTCAGCGGAACCTGCGTTTAAAGCGTCTGCGCATGCGTCGTTAG 260
ATLCV-Tz          TAATAGCCAGAGCGGCTTCAGCGGATCCTGCGTTTAATGCCTCGGCGCATGCGTCGTTAG 258
ChaMV-Ng          TAATAGCCAACGCAGCTTCAGCGGAACCTGCGTTTAACGCCTCGGCGGCTGCGTCATTAG 258
AEV-NP            TTATAGCCATTGCAGCTTCTTTAGAACCTGCATTTAGGGCTTCTGCAGCAGCATCATTAG 258
OkLCV-EG          TAACCCTAAGAGCCTCTGCCTTACTTCCTGCGTTAAGCGCTGCGGCGTAAGCGTCATTGG 258
CoLCV-SD          TAACCCTAAGAGCCTCTGCCTTACTTCCTGCGTTAAGCGCTGCGGCGTAAGCGTCATTGG 258
ToLCV-Ma          TTACATTAAGAGCCTCTGACTTACTGCCGCTGTTAAGTGCCTGTGCGTAAGCGTCATTGG 258
TbLCV-Ch          TTACATTAAGAGCCTCTGACTTACTGCCGCTGTTAAGTGCCTGGGCGTAAGCGTCATTGG 258
ToLCV-UGrev       C-CCGCCTCG-AAGGTTCG---TCGAAGGTTG-----AACTTCGACAGC----CCGTACA 241
EACMV-MW          C-CCGTCTCG-AAGGTGCG---TCGAAGGCTG-----AACTTCGACAGC----CCGTTCA 241
TYLCV-Is/PT       C-CCGCCTCG-AAGGTTCG---CCGAAGGCTG-----AACTTCGACAGC----CCATACA 241
TYLCV-JR/Szka     C-CCGCCTCG-AAGGTTCG---CCGAAGGCTG-----AACTTCGACAGC----CCATACA 241
TYLCV-OM          C-CCGTCTCG-AAGGTTCG---CCGAAGGCTG-----AACTTCGACAGC----CCATACA 241
TLCV-SD/Gez       C-CCGTCTCG-AAGGTTCG---CCGAAGGCTG-----AACTTCGACAGC----CCATACA 241
ToLCV-IN/Bg       --CCGACTT----------------------CTG-----AGCTCAATCAGC----CCATACA 184
ToLCV-TW          -----------A----------------------------TTTCCACTCA----CCGTGTA 204
                                                                        * *
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Fig.A.6.1a) continued

```
TbLCZbwV          CATTCTGGCAGCCTCCTCTAGCACTTCGTCCGTCGACCTGGAATTCTCCCCATTCAAGTG 318
TbLCZbwV-md       CATTCTGGCAGCCTCCTCTAGCACTTCGTCCGTCGACCTGGAATTCTCCCCATTCAAGTG 320
ATLCV-Tz          CATTATGGCAGCCTCCTCTAGCACTTCTGCCGTCGATCTGGAATTCCCCCCATTCGAGTG 318
ChaMV-Ng          CCTTATGGCAGCCGCCTCTAGCACTACGTCCGTCGACCTGGAACTCTCCCCACTCGATGC 318
AEV-NP            CTGTCTGTTGACCTCCTCGTGCAGATCTCCCATCGATCTGGAACTCTCCCCATTCGAGGG 318
OkLCV-EG          CGGTCTGTTGTCCTCCTCTTGCAGATCTTCCGTCGATCTGGAATTCTCCCCATTCCAGTG 318
CoLCV-SD          CGGTCTGCTGTCCTCCTCTTGCAGATCTTCCGTCAATCTGGAATTCTCCCCATTCCAGTG 318
ToLCV-Ma          CTGTCTGTTGACCCCCTCTTGCAGATCTTCCGTCGATCTGAAACTCTCCCCAATCGAGGG 318
TbLCV-Ch          CTGTCTGTTGACCCCCTCTTGCAGATCTTCCGTCGATCTGAAACTCTCCCCATTCGAGGG 318
ToLCV-UGrev       CCA--GCCGTGCTGCTGTCCCC----ACTGCCCCAGGCACAAGCAGACGTCGATCATGGA 295
EACMV-MW          GCA--GCCGTGCTGCTGTCCCC----ATTGTCCAAGGCACAAACAAGCGACGATCATGGA 295
TYLCV-Is/PT       GCA--GCCGTGCTGCTGTCCCC----ATTGTCCAAGGCACAAACAAGCGACGATCATGGA 295
TYLCV-JR/Szka     GCA--GCCGTGCTGCTGTCCCC----ATTGTCCAAGGCACAAACAAGCGACGATCATGGA 295
TYLCV-OM          GC------------------------------------------------------------ 243
TLCV-SD/Gez       GCA--ACCGTGCTGCTGTCCCC----ATTGTCCCAGGCACAAACAAGCGACGGTCATGGA 295
ToLCV-IN/Bg       GCA--AGCGTGCTGCTGTCCGC----ATTGTCCGCGGCACAAA-GGGAAAGGA--ATGGG 235
ToLCV-TW          GCTG-------CCCCC----------ACTGTCCTCGTCACAAACAAAAAGAGTTCATGGG 247


TbLCZbwV          TGTCTCCATCCTTGTCGATGTAGGACTTGACGTCGGAGCTTGATTTAGCTCCCTGA-ATG 377
TbLCZbwV-md       TGTCTCCATCCTTGTCGATGTAGGACTTGACGTCGGAGCTTGATTTAGCTCCCTGA-ATG 379
ATLCV-Tz          TATCTCCATCCTTGTCGATGTAGGACTTGACGTCGGAGCTTGATTTAGCTCCCTGA-ATG 377
ChaMV-Ng          TATCACCGTCCTTCTCGATGTAGGACTTGACGTCGGAACACGACTTAGCTCCCTGA-ACG 377
AEV-NP            TGTCTCCGTCCTTGTCGATGTAGGACTTGACGTCGGTGCTGGATTTAGCTCCCTGA-ATG 377
OkLCV-EG          TGTCTCCATCCTTGTCGATGTAGGACTTGACGTCGGAGCTGGATTTAGCTCCCTGA-ATG 377
CoLCV-SD          TGTCTCCATCCTTGTCGATGTAGGACTTGACGTCGGAGCTGGATTTAGCTCCCTGA-ATG 377
ToLCV-Ma          TGTCTCCGTCCTTGTCGATGTAGGACTTGACGTCGGAGCTGGATTTAGCTCCCTGA-ATG 377
TbLCV-Ch          TGTCTCCGTCCTTGTCGATGTAGGACTTGACGCCGGAGCTGGATTTAGCTCCCTGA-ATG 377
ToLCV-UGrev       CTTACAGGCCCATGTATCGAAAGCCCAGGATGTACAGAATGTACAGAAG-CCCTGATGTT 354
EACMV-MW          CGTTCCGGCCCATGTATCGAAAGCCCAGAATGTACAGAATGTACAGAAG-TCCTGATGTT 354
TYLCV-Is/PT       CGTACAGGCCCATGTACCGGAAGCCCAGAATATACAGAATGTATCGAAG-CCCTGATGTT 354
TYLCV-JR/Szka     CGTACAGGCCCATGTACCGGAAGCCCAGAATATACAGAATGTATCGAAG-CCCTGATGTT 354
TYLCV-OM          --TACAGGCCCATGTACCGAAAGCCCAGAATATACAGAATGTATCGAAG-CCCTGATGTT 300
TLCV-SD/Gez       CATACAGGCCCATGTACCGAAAGCCCAGAATATACAGAATGTATCGAAG-TCCTGATGTT 354
ToLCV-IN/Bg       CCAACAGGCCCATGAATCGGAAGCCCATGTTTTACAGGATGTTCAGAGG-TCCTGATGTT 294
ToLCV-TW          TGAATCGGCCCTTGTTCAGAAAGCCCAAGATGTACAGGATGTATAAAAG-CCCAGATGTT 306
                           ** *        **                        *    ** **


TbLCZbwV          TTTGGATGGAAATGTGCTGACCTGGTTGGGGATACCAGGTCGAAGAATCGG-TGATTCTT 436
TbLCZbwV-md       TTTGGATGGAAATGTGCTGACCTGGTTGGGGATACCAGGTCGAAGAATCGG-TGATTCTT 438
ATLCV-Tz          TTCGGATGGAAATGTGTTGACCTGGTTGGGGATACCAGGTCGAAGAATCTG-TTATTTTT 436
ChaMV-Ng          TTTGGATGGAAATGTGTTGATCGAGTTGGGGAGACCAGATCGAAGAATCGC-TGATTTTG 436
AEV-NP            TTCGGATGGAAATGTGCTGACCTGTTTGGGGAGACCAGGTCGAAGAATCGT-TGATTCTT 436
OkLCV-EG          TTTGGATGGAAATGTGCTGACCTATTTGGGGAGACCAGGTCGAAGAGTCTC-TGATTTTG 436
CoLCV-SD          TTTGGATGGAAATGTGTTGACCTATTTGGGGAGACCAGGTCGAAGAGTCTC-TGATTTTG 436
ToLCV-Ma          TTCGGATGGAAATGTGCTGACCTGGTTGGGGATACCAAGTCGAAGAATCTG-TTATTCTT 436
TbLCV-Ch          TTCGGATGGAAATGTGCTGACCTGGTTGGGGATACCAAGTCGAAGAATCTG-TTATTCGT 436
ToLCV-UGrev       CCTCGGGGT---TGTGAAGGCCC--------ATGTAAGGTT---CAGTCGTACGAGCAGA 400
EACMV-MW          CCTCGAGGA---TGTGAAGGCCC--------ATGTAAGGTG---CAGTCCTATGAACAGA 400
TYLCV-Is/PT       CCCCGTGGA---TGTGAAGGCCC--------ATGTAAAGTA---CAGTCTTATGAGCAAC 400
TYLCV-JR/Szka     CCCCGTGGA---TGTGAAGGCCC--------ATGTAAAGTA---CAGTCTTATGAGCAAC 400
TYLCV-OM          CCCCGTGGA---TGTGAAGGCCC--------ATGTAAAGTC---CAGTCTTATGAGCAGC 346
TLCV-SD/Gez       CCCCGTGGA---TGTGAAGGCCC--------ATGTAAGGTC---CAGTCTTATGAGCAAC 400
ToLCV-IN/Bg       CCTAGAGGC---TGTGAGGGTCC--------ATGTAAGGTC---CAATCCTTTGAGTCAA 340
ToLCV-TW          CCTCGTGGT---TGTGAAGGCCC--------ATGTAAGGTA---CAGTCATTTGAACAGC 352
                      *  *    ****  *  *         *    * **     * **     *
```

Fig.A.6.1a) continued

```
TbLCZbwV          GCAGTTGAATTTTCCCTCGAA---CTGAATAAGCACGTGGAGATGAGGTTCCC-CATTTT 492
TbLCZbwV-md       GCAGTTGAATTTTCCCTCGAA---CTGAATAAGCACGTGGAGATGAGGTTCCC-CATTTT 494
ATLCV-Tz          GCAGTTGAATTTTCCCTCGAA---CTGAATAAGCACGTGGAGATGAGGCTCCC-CATCTT 492
ChaMV-Ng          GCACTTGTATTTGCCCTCGAA---CTGAATGAGGGCATGCAGATGAGGTTGCC-CATCCT 492
AEV-NP            GCACTGATATTTCCCTTCGAA---CTGGATGAGCACATGGAGATGAGGGCTCC-CATCTT 492
OkLCV-EG          GCACTTGAACTTCCCTTCGAA---CTGGAGAAGCACATGTAGATGAGGTTGGC-CATCCT 492
CoLCV-SD          GCACTTGAACTTCCCTTCGAA---CTGGAGAAGCACATGTAGATGAGGTTGGC-CATCCT 492
ToLCV-Ma          GCACTGGTATTTCCCTTCGAA---CTGGATAAGCACGTGGAGATGAGGGCTCC-CATCTT 492
TbLCV-Ch          GCAGACGTATTTCCCTTCGAA---CTGGATAAGCACGTGGAGATGAGGGCTCC-CATCTT 492
ToLCV-UGrev       GAGATGATGTTAAGCATACTG---GTGTTGTCCGTTGTGTTAGTGATGTTACTTCGTGGG 457
EACMV-MW          GAGACGATGTTAAGCACACCG---GTGCTGTGCGTTGTGTTAGTGATGTTACT-CGTGGT 456
TYLCV-Is/PT       GGGATGATATTAAGCATACTG---GTATTGTTCGTTGTGTTAGTGATGTTACT-CGTGGA 456
TYLCV-JR/Szka     GGGATGATATTAAGCATACTG---GTATTGTTCGTTGTGTTAGTGATGTTACT-CGTGGA 456
TYLCV-OM          GGGATGATATTAAGCACACTG---GTGTTGTTCGTTGTGTTAGTGATGTTACT-CGTGGA 402
TLCV-SD/Gez       GGGATGATATTAAGCATACTG---GTATTGTTCGTTGTGTTAGTGATGTTACT-CGTGGA 456
ToLCV-IN/Bg       GACACGATATAATTCATATAG---GGAAGGTCATGTGTATTAGTGATGTCACT-CGCGGT 396
ToLCV-TW          GTCATGATATAGCCCATGTGG---GCAAGGTAATATGTGTGTCCGATGTCACA-CGAGGT 408
                  *       *     *                              ** *      *

TbLCZbwV          CGTGTAGCTCTCTGCAGATTTTGATGTATTTTTTATTGGTGGGTGTTTCAAGGTTTAATA 552
TbLCZbwV-md       CGTGTAGCTCTCTGCAGATTTTGATGTATTTTTTATTTGTGGGTGTTTCAAGGTTTAATA 554
ATLCV-Tz          CGTGTAGTTCTCTGCAAATTTTGATGTATTTTTTATTTGTTGGAGTATCAGTATTTATTA 552
ChaMV-Ng          CGTGTAACTCACGACAAACTTTGATGTATTTCTGGTTCGTGGGCGTGCGTAAGCTTCGTA 552
AEV-NP            CGTGTAGTTCTCTGCAGATCTTGATGTATTTTTTGTTTGTTGGGGGTTGGAGATTTAATA 552
OkLCV-EG          CGTGAAGCTCTCTGCAGATCTTGATATATTTCTTGTTTGAAGCTGTGCTTATTTGCTGTA 552
CoLCV-SD          CGTGAAGCTCTCTGCAGATCTTGATATATTTCTTGTTTGAAGCTGTGCTTATTTGTTGAA 552
ToLCV-Ma          CGTGAAGCTCTCTGCAGATTTTGATATATTTCTTGTTGGTTGGGGTGTTTAGGTTTTGTA 552
TbLCV-Ch          CGTGAAGCTCTCTGCAGATTTTGATATATTTCTTATTAGTTGGTGTCTCTAGGGCTTGTA 552
ToLCV-UGrev       TCGGGTA-TTACACATAGGGTTGGCAAGAGGTTCTGTGTGAAGTCCATTTACATTATAGG 516
EACMV-MW          TCGGGTA-TTACTCATAGGGTAGGGAAGAGATTTTGTGTGAAGTCAATATATGTTTTAGG 515
TYLCV-Is/PT       TCTGGAA-TTACTCACAGAGTGGGTAAGAGGTTCTGTGTTAAATCGATATATTTTTTAGG 515
TYLCV-JR/Szka     TCTGGAA-TTACTCACAGAGTGGGTAAGAGGTTCTGTGTTAAATCGATATATTTTTTAGG 515
TYLCV-OM          TCTGGAA-TTACTCACAGAGTCGGTAAGAGGTTCTGTGTTAAATCGATATATTTTTTAGG 461
TLCV-SD/Gez       TCCGGAA-TTACCCACAGAGTGGGGAAGAGGTTCTGTGTTAAATCGATATATTTTTTAGG 515
ToLCV-IN/Bg       ACGGGGT-TAACACATAGAGTTGGTAAGCGGTTTTGTGTCAAGTCAGTATACGTTTTGGG 455
ToLCV-TW          AATGGGC-TGACCCATCGTGTTGGGAAGAGGTTTTGTGTTAAGTCCATTTATGTGTTGGG 467
                     *    *  *        *           *

TbLCZbwV       ATT-- 555
TbLCZbwV-md    ATT-- 557
ATLCV-Tz       ATT-- 555
ChaMV-Ng       ATT-- 555
AEV-NP         ATT-- 555
OkLCV-EG       TTT-- 555
CoLCV-SD       GTT-- 555
ToLCV-Ma       ATT-- 555
TbLCV-Ch       ATT-- 555
ToLCV-UGrev    GAAAA 521
EACMV-MW       AAAGA 520
TYLCV-Is/PT    TAAAG 520
TYLCV-JR/Szka  TAAAG 520
TYLCV-OM       TAAAG 466
TLCV-SD/Gez    TAAAG 520
ToLCV-IN/Bg    TAAAA 460
ToLCV-TW       TAAGA 472
```

Figure A.6.1b) ToLCV-Ugf

```
ToLCV-Ugfseq   ----------------------ACGACTA-------TCAC---------------------- 11
ATLCV-TZ       ----------------------ACGACTA-------TCCCCTTCA--ATCACAATACTCAT 30
TbLCZwV-md     ----------------------GTCACTAGGGGTGGTGGTCTTACGCATCGCACTGGTAAA 39
TbLCZwV        ----------------------ACGACTA-------TCGCCTTCT--ATTACAATACTATT 30
TCSV-SA        ----------------------ACGACTA-------TCGCCCTCT--ATTACAATACTATT 30
CMV-Ng         ----------------------CCGACTA-------TCACCCTCG--ACTATTATACTACT 30
TbLCV-Ch       ----------------------ACGACTC-------TCTCCCTCT--ATGACAATACTGAT 30
ToLCV-Ma       ----------------------ACGACTC-------TCTCCCTCT--ATGACAATACTATT 30
SCLV-Jr        ----------------------ACGACTC-------TCGCCCTCT--ATTACAATACTATT 30
ToLCV-Ph       ----------------------TCGACTA-------TCGC---------------------- 11
CoLCV-SD       ----------------------TCTGCTT-------TCCCCTTCA--ATCACTATACTAAT 30
OkLCV-EG       ------------------------------------------------------------
ToLCV-TW       ----------------------CTTCCCTGTACGACTCTCTCCCTCTACCACAATACTCAT 39
ToLCV-Au       ---------------------------------------------AATCAATAGAAAAAAA 16


ToLCV-Ugfseq   ------------------------ACGACTA-------TCA---CTCA-----GACACCC 32
ATLCV-TZ       GGGTCTTAAT-----GGCCGCGCAGCGGTAGTTAGA--ACA---TTCTGT---GCCGCCC 77
TbLCZwV-md     CGCTTTTGTATT---AGCCGCGCAGCGGCACCACAC--ACA---TTCTCA---GAGACCC 88
TbLCZwV        GGGCCTCCAA-----GGCCGCGCAGCGGCACCACAC--ACA---TTCTCA---GAGACCC 77
TCSV-SA        GGGTCTCCAA-----GGCCGCGCAGCGGCACCACAC--ACA---TTCTCA---GAGACCC 77
CMV-Ng         CGGCCTCCAC-----GGCCGCGCAGCGGCATCCATC--ACG---TTCGCC---GATGCCC 77
TbLCV-Ch       GGGTCTCCAT-----GGCCGCGCAGCGGCATGCCTT--ACA---TTATCAA--GCAGCCC 78
ToLCV-Ma       GGGTCTCCAA-----GGCCGCGCAGCGGCATCCCTG--ACG---TTCTTA---GAAACCC 77
SCLV-Jr        AGGTCTCCAT-----GGCCGCGCAGCAT--TCCCGT--ACA---TTATCA---GCAGCCC 75
ToLCV-Ph       ----------------------TCGACTA----------TCG---CTCG-----GACACCC 32
CoLCV-SD       CGGTCTACTT-----GGCCGCGCAGCGGCATCAACG--ACG---TTCTCC---GCCGCCC 77
OkLCV-EG       ---TCTGCTT--------------TCCCCTTCA--A--TCA---CTAT-----GCCGCCC 31
ToLCV-TW       GGGTCTCCAT-----GGCCGCGCAGCGGAATCCCTC--ACG---TTCTCG---GCAACCC 86
ToLCV-Au       AAGTGGTTAA--------GGCACTGTGACAAATAATTG-----ACTTGAAGGACCCACTT 63


ToLCV-Ugfseq   AACATTCAAGTTCCTCTGGAACTTGATCAAATGAAGAAGA-AAG--AAAAGGAGAAACAT 89
ATLCV-TZ       ACTCTTCAAGTTCCTCTGGAACTTGATCGAAAGAAGAACA-TAA--GAAAGGAGAAACAT 134
TbLCZwV-md     ACTCTTCAAGTCCCTCAGGGACTTGATCAAAAGAAGAAGA-AGA--GAAAGGAGAAATAT 145
TbLCZwV        ACCCTTCAAGTTCCTCAGGGACTTGATCAAAAGAAGAAGA-AGA--GAAAGGAGAAATAT 134
TCSV-SA        ACTCTTCAAGTTCCTCAGGGACTTGATCAAAAGAAGATGA-AGA--AAAAGGAGAAATAT 134
CMV-Ng         ATCGTTGAAGTTCATCAGGAACCTGAGTGAAGGAGGAAGC-ACA--GAAGGGAGAAACAT 134
TbLCV-Ch       ACTCTTCAAGTTCTTCGGGAACTTGATCAAATGATGAAGA-TAA--AAAAGGAGAAACAA 135
ToLCV-Ma       ACTCTTCAAGTTCTTCGGGAACTTGATCGAAAGAAGAAGA-AAG--AAAAGGACAAACAA 134
SCLV-Jr        AAACTTCAAGTTCTTCCGGAACTTGATCAAAAGAAGAAGA-AGA--AAAAGGAGAAACAT 132
ToLCV-Ph       ATTCTTCAAGTTCTTTTGGAACTTGATTGAAAGATGAAGA-AGG--AAAAGGACTAACAA 89
CoLCV-SD       ATTCTTCAAGTTCTTCTGGAACTTGGTCGAACGAAGAAGA-CAA--AAAAGGAGAAACAT 134
OkLCV-EG       ATTCTTCAAGTTCTTCGGGAACTTGGTCGAACGAAGAAGA-CAA--AAAAGGAGAAACAT 88
ToLCV-TW       ATTCTACAAGTTCTACTGGAACTTGATCAAATGAAGAAGA-ATT--AAAAGGAGAAATAT 143
ToLCV-Au       ACTGCTGGGTCCGTGTTAATTATTTATCCAAAAATAAAAAGAAA--AAGAAAATAGATAT 121
               *                     *     **  *  *          * *  *   * *


ToLCV-Ugfseq   A-TCCTTCAACGGAGGTGTAAAAATCTTATCTAAATTACA-TTTAAATTATGATACTGAA 147
ATLCV-TZ       AATCCTCCAACGGAGGTGTAAAAATCCTATCTAAATTACATTTTAAATTATGATACTGAA 194
TbLCZwV-md     AAGGCTCTGGGGGGAGCCTGGAAAATCCTATCTAAATTAGTCTTTAAATTATGATACTGAA 205
TbLCVZbw       AAGGCTCTGGGGGGAGCCTGGAAAATCCTATCTAAATTAGTCTTTAAATTATGATACTGAA 194
TCSV-SA        AAGGCTCTGGGGGGAGCCTGGAAAATCCTATCTAAATTAGTCTTTAAATTATGATACTGAA 194
CMV-Ng         AAACCTCCGGAGGAGCTTGGAAGATCTTATCCAAATTAGCCTTGATATTATGATATTGAA 194
TbLCVCh        AA-CCTCTAAAGGAGGTGCAAAAATCCTATCTAAATTAGCATTTAAATTATGAAATTGTA 194
ToLCVMa        AAACCTCTACAGGAGGTGCAAAAATCCTATCTAAATTAGAATTTAAATTATGAAATTGTA 194
SCLV-Japan     AA-CCTCTAAAGGAGGCGTAAAAATCCTATCTAAATTACTATTTAAATTATGAAATTGTA 191
ToLCVPh        AAACCTCTAAAGGAGGTGCAAAAATCCTATCTAAATTTGCACTTAAATTATGAAACTGTA 149
CoLCV-Sudan    AAGGAGCTGGAGGCTCCTGAAAAATCCTCTCTAAATTACTATTTAAATTATGAAATTGTA 194
OkLCV-EG       AAGGAGCTGGAGGCTCCTGAAAAATCCTCTCTAAATTACTATTTAAATTATGAAATTGTA 148
ToLCV-TW       AAACCTCTAATGGAGGTGTAAAAATCCTATCTAAATTACTATTTAAATTATGAAACTGTA 203
ToLCV-Au       GCAGTTAAAATTAGTTACCACAATAACTAATTGATTTACCATTTACCCAA--ATGGTAAA 179
                              *     *     * **     * *    *  *   *  *
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Fig.A.6.1b) continued

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ToLCV-Ugfseq   AAATAAAATCCTTAGGGAGT---TTCTCCCTAATAA------TAGCCAA---AGCGGCTT 195
ATLCV-TZ       AAATGAAATCTTTAGGGAGT---TTCTCCCTAATAA------TAGCCAG---AGCGGCTT 242
TbLCZwV-md     AAATAAAATCTTTAGGGAGT---TTTTCCCTAATAA------TAGCCAT---AGCTGCTT 253
TbLCZwV        AAATAAAATCTTTAGGGAGT---TTTTCCCTAATAA------TAGCCAT---AGCTGCTT 242
TCSV-SA        AAATAAAATCTTTAGGGAGT---TTTTCCCTAATAA------TAGCCAT---AGCGGCTT 242
CMV-Ng         ATATATAATCTTTGGGGAGT---TTCTCCTTAATAA------TAGCCAA---CGCAGCTT 242
TbLCV-Ch       AAACAAAATCTTTAGGAGCT---AACTCCTTAATTA------CATTAAG---AGCCTCTG 242
ToLCV-Ma       AAACAAAATCTTTAGGAGCT---AACTCCTTAATTA------CATTAAG---AGCCTCTG 242
SCLV-Jr        AAACAAAATCTTTAGGGGCT---AATTCCCTAATTA------CATTAAG---AGCCTCTG 239
ToLCV-Ph       AAACAAAATCTTTTGGTGCT---AATTCCCTAATTA------CATTAAG---AGCCTCTG 197
CoLCV-SD       AAACAAAATCTTTGGGAGCT---AACTCCCTTATAA------CCCTAAG---AGCCTCTG 242
OkLCV-EG       AAACAAAATCTTTGGGAGCT---AACTCCCTTATAA------CCCTAAG---AGCCTCTG 196
ToLCV-TW       AAACAAAATCTTTGGGAGCT---TTCTCCCTTAATA------TATTGAG---GGCCTCAG 251
ToLCV-Au       TATTGAGACACCGATAG-GTAAATTGTCCCCAATTGAATCGGTGTCTATTGGGGACAATG 238
                      *           *      * *   *             *     *

ToLCV-Ugfseq   CA--------GCGGAACCTGCGTTTAA----TGCCTCGG-CGGCTGCGT-CGTTAGCATT 241
ATLCV-TZ       CA--------GCGGATCCTGCGTTTAA----TGCCTCGG-CGCATGCGT-CGTTAGCATT 288
TbLCZwV-md     CA--------GCGGAACCTGCGTTTAA----AGCGTCTG-CGCATGCGT-CGTTAGCATT 299
TbLCZwV        CA--------GCGGAACCTGCGTTTAA----AGCGTCGG-CGCATGCGT-CGTTAGCATT 288
TCSV-SA        CA--------GCGGAACCTGCGTTTAA----AGCGTCGG-CGCATGCGT-CGTTAGCATT 288
CMV-Ng         CA--------GCGGAACCTGCGTTTAA----CGCCTCGG-CGGCTGCGT-CATTAGCCTT 288
TbLCV-Ch       AC--------TTACTGCCGCTGTTAAG----TGCCTGGG-CGTAAGCGT-CATTGGCTGT 288
ToLCV-Ma       AC--------TTACTGCCGCTGTTAAG----TGCCTGTG-CGTAAGCGT-CATTGGCTGT 288
SCLV-Jr        AC--------TTACTGCCGCTGTTAAG----TGCCTTGG-CGTAAGCGT-CGTTGGCCGA 285
ToLCV-Ph       AC--------TTACTTCCTGTGTTAAT----CGCTTTGG-CGTAAGCGT-CATTGGCTGA 243
CoLCV-SD       CC--------TTACTTCCTGCGTTAAG----CGCTGCGG-CGTAAGCGT-CATTGGCGGT 288
OkLCV-EG       CC--------TTACTTCCTGCGTTAAG----CGCTGCGG-CGTAAGCGT-CATTGGCGGT 242
ToLCV-TW       CT--------TTGGACCCTGAATTGAT----TGCCTCGG-CATATGCGT-CGTTGGCAGA 297
ToLCV-Au       GG---TATCATCGTTTTTTGGACTAAA---ATGCCCCTGTCAT-TGTGTCTGTGAGGCGC 291
                                 *        **    *      *  *   *  *

ToLCV-Ugfseq   CTGGCAGCCTCCT-----CTAGCACTTCGTCC-GTCGACCTGAAATTCTCCC-ATTCGA- 293
ATLCV-TZ       ATGGCAGCCTCCT-----CTAGCACTTCTGCC-GTCGATCTGGAATTCCCCCCATTCGA- 341
TbLCZwV-md     CTGGCAGCCTCCT-----CTAGCACTTCGTCC-GTCGACCTGGAATTCTCCCCATTCAA- 352
TbLCZwV        CTGGCAGCCTCCT-----CTAGCACTTCGTCC-GTCGACCTGGAATTCTCCCCATTCAA- 341
TCSV-SA        TTGGCAGCCTCCT-----CTAGCACTTCTGCC-GTCGACCTGGAATTCTCCCCATTCAA- 341
CMV-Ng         ATGGCAGCCGCCT-----CTAGCACTACGTCC-GTCGACCTGGAACTCTCCCCACTCGA- 341
TbLCV-Ch       CTGTTGACCCCCT-----CTTGCAGATCTTCC-GTCGATCTGAAACTCTCCCCATTCGA- 341
ToLCV-Ma       CTGTTGACCCCCT-----CTTGCAGATCTTCC-GTCGATCTGAAACTCTCCCCAATCGA- 341
SCLV-Jr        TTGCTGACCCCCT-----CTTGCAGATCGTCC-GTCGATCTGAAACTCTCCCCACTCGA- 338
ToLCV-Ph       TTGTTGTCCCCCT-----CTTGCAGATCTTCC-GTCGACCTGAAACTCTCCCCATTCGA- 296
CoLCV-SD       CTGCTGTCCTCCT-----CTTGCAGATCTTCC-GTCAATCTGGAATTCTCCCCATTCCA- 341
OkLCV-EG       CTGTTGTCCTCCT-----CTTGCAGATCTTCC-GTCGATCTGGAATTCTCCCCATTCCA- 295
ToLCV-TW       TTGGCAACCTCCT-----CTAGCTGATCTTCC-ATCGACCTGGAAAATTCCATGATCAA- 350
ToLCV-Au       GTCGTAGTGCGCT-----GCAAAAGTAAGACCTGCAACTTTCCCAAACTGAAATTCCGGC 346
                *         **                **        *   *   *

ToLCV-Ugfseq   --------GGGTGTCTCCATCCTTGTCGATGTAGGACTTGACGTCGGAGCTGGAT--TTA 343
ATLCV-TZ       --------GTGTATCTCCATCCTTGTCGATGTAGGACTTGACGTCGGAGCTTGAT--TTA 391
TbLCZwV-md     --------GTGTGTCTCCATCCTTGTCGATGTAGGACTTGACGTCGGAGCTTGAT--TTA 402
TbLCZwV        --------GTGTGTCTCCATCCTTGTCGATGTAGGACTTGACGTCGGAGCTTGAT--TTA 391
TCSV-SA        --------GTGTGTCTCCATCCTTGTCGATGTAGGACTTGACGTCGGAGCTGGAT--TTA 391
CMV-Ng         --------TGCTATCACCGTCCTTCTCGATGTAGGACTTGACGTCGGAACACGAC--TTA 391
TbLCV-Ch       --------GGGTGTCTCCGTCCTTGTCGATGTAGGACTTGACGCCGGAGCTGGAT--TTA 391
ToLCV-Ma       --------GGGTGTCTCCGTCCTTGTCGATGTAGGACTTGACGTCGGAGCTGGAT--TTA 391
SCLV-Jr        --------GGGTGTCTCCGTCCTTGTCGATATAGGACTTGACGTCGGAGCTGGAT--TTA 388
ToLCV-Ph       --------GGGTGTCTCCGTCCTTGTCGATATAGGACTTGACGTCGGAGCTGGAT--TTA 346
CoLCV-SD       --------GTGTGTCTCCATCCTTGTCGATGTAGGACTTGACGTCGGAGCTGGAT--TTA 391
OkLCV-EG       --------GTGTGTCTCCATCCTTGTCGATGTAGGACTTGACGTCGGAGCTGGAT--TTA 345
ToLCV-TW       --------GCACGTCTCCGTCTTTCTCCATATAGGTTTTGACATCTGTTGAGCTC--TTA 400
ToLCV-Au       ATAACT--TC-CGATTCCGAGCTCCG-ATTGCAACACGCGCGGGCGGTGGGACATG-TTA 401
                       **    *      *  *      *    * *          ***
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Fig.A.6.1b) continued

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ToLCV-Ugfseq   ACTCCCTGAATGTT------------TGGATGGAAATG----------TGCTGACCTGGT 381
ATLCV-TZ       GCTCCCTGAATGTT------------CGGATGGAAATG----------TGTTGACCTGGT 429
TbLCZwV-md     GCTCCCTGAATGTT------------TGGATGGAAATG----------TGCTGACCTGGT 440
TbLCZwV        GCTCCCTGAATGTT------------TGGATGGAAATG----------TGCTGACCTGGT 429
TCSV-SA        GCTCCCTGAATGTT------------TGGATGGAAATG----------TGCTGACCTGTT 429
CMV-Ng         GCTCCCTGAACGTT------------TGGATGGAAATG----------TGTTGATCGAGT 429
TbLCV-Ch       GCTCCCTGAATGTT------------CGGATGGAAATG----------TGCTGACCTGGT 429
ToLCV-Ma       GCTCCCTGAATGTT------------CGGATGGAAATG----------TGCTGACCTGGT 429
SCLV-Jr        GCTCCCTGAATGTT------------CGGATGGAAATG----------TGCTGACCTGGT 426
ToLCV-Ph       GCTCCCTGAATGTT------------CGGATGGAAATG----------TGCTGACCTGGT 384
CoLCV-SD       GCTCCCTGAATGTT------------TGGATGGAAATG----------TGTTGACCTATT 429
OkLCV-EG       GCTCCCTGAATGTT------------TGGATGGAAATG----------TGCTGACCTATT 383
ToLCV-TW       GCTCCCTGAATGTT------------CGGATGGAAATG----------TGCTGACCTGGT 438
ToLCV-Au       AATGCCCAAAC----------CACTACGCTAGGCAGCCTTAGCTCCGCACGTAGCTTAAC 451
                 * **  **               *   ** *              *

ToLCV-Ugfseq   TGGGGATACCAGGTCGAAGA-ATCGGTGATTCTTGCAGG-TGTATTTGCCTTCGA-CTG- 437
ATLCV-TZ       TGGGGATACCAGGTCGAAGA-ATCTGTTATTTTTGCAGT-TGAATTTTCCCTCGAACTG- 486
TbLCZwV-md     TGGGGATACCAGGTCGAAGA-ATCGGTGATTCTTGCAGT-TGAATTTTCCCTCGAACTG- 497
TbLCZwV        TGGGGATACCAGGTCGAAGA-ATCGGTGATTCTTGCAGT-TGAATTTTCCCTCGAACTG- 486
TCSV-SA        TGGGGATACCAGGTCGAAGA-ATCGGTGATTCTTGCAGT-TGAATTTTCTCTCGAACTG- 486
CMV-Ng         TGGGGAGACCAGATCGAAGA-ATCGCTGATTTTGGCACT-TGTATTTGCCCTCGAACTG- 486
TbLCV-Ch       TGGGGATACCAAGTCGAAGA-ATCTGTTATTCGTGCAGA-CGTATTTCCCTTCGAACTG- 486
ToLCV-Ma       TGGGGATACCAAGTCGAAGA-ATCTGTTATTCTTGCACT-GGTATTTCCCTTCGAACTG- 486
SCLV-Jr        TGGGGATACCAAGTCGAAGA-ATCTGTTATTCTTGCACT-GGTATTTCCCTTCGAACTG- 483
ToLCV-Ph       TGGGGATACCAAGTCGAAGA-ATCGCTGGATCTGGCACT-GGTATTTTCTCTCGAATTG- 441
CoLCV-SD       TGGGGAGACCAGGTCGAAGA-GTCTCTGATTTTGGCACT-TGAACTTCCCTTCGAACTG- 486
OkLCV-EG       TGGGGAGACCAGGTCGAAGA-GTCTCTGATTTTGGCACT-TGAACTTCCCTTCGAACTG- 440
ToLCV-TW       TGGGGATGTGAGGTCGAAGA-ATCTGTTATTTTTACATT-GGAATTTTCCTTCGAATTGG 496
ToLCV-Au       GCCTCTTCGGAGCTCAACGACATCCATGGTGGTTCTGGT-GGTACGGGCACC-------- 502
                         *  ** * **  *   *             *
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Annex 7:

Figure A.7.1: CLUSTAL W (1.82) multiple sequence alignment for TYLCV-UG Intergenic region (IR) compared to TYLCV-EG, TYLCV-CU, TYLCV-JM, TYLCV-MX, TYLCV-LB and OYVMV-PK for out group rooting: (-) indicates no consensus, while areas of maximumconsensus are marked with asterisks (*)

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TYLCV-EG        ------------------------------------ATCGGTGTCCCTCAA------AG 17
TYLCV-LB        ------------------------------------ATCGGTGTCCCTCAA------AG 17
TYLCV-UG        ------------------------------------ATCGGTGTCCCTCAA------AG 17
TYLCV-CU        ------------------------------------ATCGGTGTCCCTCAA------AG 17
TYLCV-JM        ------------------------------------ATCGGTGTCCCTCAA------AG 17
TYLCV-MX        ------------------------------------ATCGGTGTCCCTCAA------AG 17
TYLCV-US/S      ------------------------------------ATCGGTGTCCCTCAA------AG 17
OYVMV-PK        ------------------------------------ATCGGTACCC---AGA--TCTAG 18
                                                     ******  **   *       **

TYLCV-EG        CTCTATGGCAATCGGTGTATCGGTGTCTTACTTATACCTGGACACCTAATGGCTATTTG- 76
TYLCV-LB        CTCTATGGCAATCGGTGTATCGGTGTCTTACTTATACCTGGACACCAAATGGCATTTTG- 76
TYLCV-UG        CTCTATGGCAATCGGTGTATCGGTGTCTTACTTATACCTGGACACCTAATGGCTATTTG- 76
TYLCV-CU        CTCTATGGCAATCGGTGTATCGGTGTCTTACTTATACCTGGACACCTAATGGCTATTTG- 76
TYLCV-JM        CTCTATGGCAATCGGTGTATCGGTGTCTTACTTATACCCGGATACCTAATGGCTATTTG- 76
TYLCV-MX        CTCTATGGCAATCGGTGTATCGGTGTCTTACTTATACCTGGATACCTAATGGCTATTTG- 76
TYLCV-US/S      CTCTATGGCAATCGGTGTATCGGTGTCTTACTTATACCTGGATACCTAATGGCTATTTG- 76
OYVMV-PK        TCTTATGTCAATTGGTG-AACGGTACCCTATATATAG-TGGGTACTGAATGGCAATATTT 76
                   **** **** **** * ****  *  *  ****   **  **  ******  * *

TYLCV-EG        GTAATTTCATAAATGTTC-ATTGCAATTCAAAATTCAAAATTCAAAAATCAAATCATTAA 135
TYLCV-LB        GTAATTTAGTAAAAGTAC-ATTGCAATTCAAAATTCAAAATTCAAAAATCAAATCATTAA 135
TYLCV-UG        GTAATTTCATAAATGTTC-ATTGCAATTCAAAATTCAAAATTCAAAAATCAAATCATTAA 135
TYLCV-CU        GTAATTTCATAAATGTTC-ATTGCAATTCAAAATTCAAAATTCAAAAATCAAATCATTAA 135
TYLCV-JM        GTAATTTCATAAATGTTC-ATTGCAATTCAAAATTCAAAATTCAAAAATCAAATCATTAA 135
TYLCV-MX        GTAATTTCATAAATGTTC-ATTGCAATTCAAAATTCAAAATTCAAAAATCAAATCATTAA 135
TYLCV-US/S      GTAATTTCATAAATGTTC-ATTGCAATTCAAAATTCAAAATTCAAAAATCAAATCATTAA 135
OYVMV-PK        GTAATTATGAAAA----------TAAATTCAAAATCCTCACGCTCCAA-----------AA 116
                ******    ***           ********** *  *      **           **

TYLCV-EG        AGCGGCCATCCGTATAATATTACCGGATGGCCGCGCCTTTTCCTTTTATGTGGTCCCCAC 195
TYLCV-LB        AGCGGCCATCCGTATAATATTACCGGATGGCC-CGCCTTTTCCTTTTGTGTGGTCCCCAC 194
TYLCV-UG        AGCGGGCATCCGTATAATATTACCGGATGGCCGCGCCTTTTCCTTTTATGTGGTCCCCAC 195
TYLCV-CU        AGCGGTCATCCGTATAATATTACCGGATGGCCGCGCCTTTTCCTTTTATGTGGTCCCCAC 195
TYLCV-JM        AGCGGTCATCCGTATAATATTACCGGATGGCCGCGCCTTTTCCTTTTATGTGGTCCCCAC 195
TYLCV-MX        AGCGGTCATCCGTATAATATTACCGGATGGCCGCGCCTTTTCCTTTTATGTGGTCCCCAC 195
TYLCV-US/S      AGCGGTCATCCGTATAATATTACCGGATGGCCGCGCCTTTTCCTTTTATATGGTCCCCAC 195
OYVMV-PK        AGCGGCCATCCGTATAATATTACCGGATGGCCGCGCGATTTTT----------TTTATTG 166
                ***** ************************ ***  ***            *

TYLCV-EG        GAGGGTTACACAGACGTCACTGTCAACCAATCAAATTGCATCCTCAAACGTTAGATAAGT 255
TYLCV-LB        GAGGGTTACACAGACGTCACTGTCAACCAATCAAATTGCATCCTCAAACGTTAGATAAGT 254
TYLCV-UG        GAGGGTTACACAGACGTCACTGTCAACCAATCAAATTGCATCCTCAAACGTTAGATAAGT 255
TYLCV-CU        GAGGGTTACACAAACGTCACTGTCAACCAATCAAATTGCATCCTCAAACGTTAGATAAGT 255
TYLCV-JM        GAGGGTTACACAGACGTCACTGTCAACCAATCAAATTGCATCCTCAAACGTTAGATAAGT 255
TYLCV-MX        GATGGTTACACAGACGTCACTGTCAACCAATCAAATTGCATCCTCAAACGTTAGATAAGT 255
TYLCV-US/S      GAGGGTTACACAGACGTCACTGTCAACCAATCAAATTGCATCCTCAAACGTTAGATAAGG 255
OYVMV-PK        GTGGGTCCAG--------------AACGCGCGACGATGCAGACTCAAAGCTTAGATAACG 212
                * ****                  ***     *   ****  ******  ********

TYLCV-EG        GTGCATTTGTCTTTATATACTT---------------------------------------- 277
TYLCV-LB        GTTCATTTGTCTTTATATACTT---------------------------------------- 276
TYLCV-UG        GTGCATTTGTCTTTATATACTT---------------------------------------- 277
TYLCV-CU        GTTCATTTGTCTTTATATACTT---------------------------------------- 277
TYLCV-JM        GTTCATTTGTCTTTATATACTT---------------------------------------- 277
TYLCV-MX        GTTCATTTGTCTTTATATACTT---------------------------------------- 277
TYLCV-US/S      GTTCATTTGTCTTTATATACTT---------------------------------------- 277
OYVMV-PK        CTCCT-TTGGCTATAAGTACTT---------------------------------------- 233
                 * *  *** ** **  *****
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9 CURRICULUM VITAE

Name and Address	Ssekyewa Charles Uganda Martyrs University P.O.BOX 5498, Kampala, Uganda Tel. +256-382-410611 Mob. +256-772-517158 Fax.+ 256-382-410100 e-mail: cssekyewa@umu.ac.ug
Sex	Male
Date of Birth	28th March 1961
Place of Birth	Kalisizo, Uganda
Nationality	Ugandan
Marital Status	Married with four children: 2 girls and 2 boys

ACADEMIC QUALIFICATIONS

Degree	Year	Institution	Specialization
MSc.	1990-1991	University of London, Wye College	Tropical and sub-tropical horticulture and crop science Research on Collar rot disease of Passion fruits
BSc.	1982-1985	Makerere University	B.Sc. (Botany, Geography, Zoology) Research on Taxonomy of Leguminous plants

ADDITIONAL PROFFESIONAL TRAINING

Field	**Year**	**Institution**	**Duration**
Organic agriculture Certification course	2004	Grolink AB, Sweden	3 days
Organic agriculture	2003	UMU/EPOPA-Grolink (OSDT), Uganda	2 weeks
Refresher course in Agriculture	2001	Uganda Martyrs University	5 days
Radiation safety for Radiation workers			
Training and hands on molecular virology	1998	University of Wisconsin	2 months
Research proposal writing course	1998	Makerere University	11 days
Vegetables Virology	1996	AVRDC, Tanzania	3 weeks
Participatory Integrated Pest Management	1995	Makerere University	4 days
Field Survey methodology	1995	GTZ-IPM, Kenya	1 week
Vegetable diseases	1994	AVRDC, Tanzania	1 month
Management and organization of biological control programs	1994	GTZ/IITA/COMO, Kenya	2 weeks
Identification of *Fusarium* spp. Fungi	1993	Pennyslavania State Univeristy, USA	1 week
Vegetable production	1989	International Agricultural Center, Netherlands	3 mths, 2 wks
Crop protection	1988-1989	UGA/87/003/FAO/UNDP, Horticulture Project, Uganda	1 week series
Seed production	1988	PNAP, Rwanda	2 weeks

EMPLOYMENT RECORD

Position	**Duration**	**Grade**	**Institution**	**Responsibility**
Dean Faculty of Agriculture	September 2000- 2005	Senior Lecturer	Uganda Martyrs University	Administration Training, Research, Outreach
Coordinator, GTZ-IPM Horticulture	1994-2000	UR5	National Agricultural Research Organization	Administration Research, Training, Outreach
Research Officer	1993-1994	U5-U3	Ministry of Agriculture, Research Division	Research, Training, Outreach
Counterpart to Pathology Expert, UGA/87/003/FAO/UNDP Project	1989-1993	U5-3	Ministry of Agriculture, Research Division	Research, Training, Outreach
Scientific Officer	1986-1989	U5-3	Ministry of Agriculture, Research Division	Research

SCIENTIFIC CONTRIBUTIONS

1-Responsibilities and Achievements

Training

Organic agriculture training, research, membership to the Uganda Organic Agriculture Certification Company (UgoCert) Certification Committee and Secretary to the Board of Directors of the same certification company.

Training in Organic Agriculture under the Organic Sector Development Training of the Export Promotion of Organic Products from Africa, EPOPA funded by the Swidish Development Agency (SIDA), and organization of similar local training

Facilitated during the training of trainers and especially extension workers, Horticulture training programs, UGA/87/003/FAO/UNDP Project

Research Experience

Research Fellow of AFP Rothamsted, in collaboration with Dr. Katherine Steel, University of Bangor, UK, (2007): the study will focus on identification of tomato yellow leaf curl viruses in all tomato growing agro-ecologies of Uganda, other than the eight districts covered during this study, evaluation of selected tomato lines against tomato yellow leaf curl viruses, dissemination of elite tomato germplasm to farmers in Lake Victoria Basin

Research on knowledge gaps along the export market chain of organic exports in Uganda. In collaboration with the National Organic Agriculture Movement of Uganda, (2006)

Research on the impact (knowledge, attitude, income, nutrition) of the vegetable gardening activity in Universal Primary Education Schools on the school community and on surrounding villages (I@Mak.com 2005 Rockfeller Funded Project): the objective of this activity is to impact agricultural skills to pupils so that those who may drop out of school after Primary seven will be able to use the skill

Research, on first identification of tomato viruses in Uganda (1997-2006), as part of PhD research work

Evaluation of three tomato lines, UMU1, UMU2, and UMU3 for their performance under organic farming systems, with no use of synthetic chemicals (2004-2005)

Participation in regional evaluation of AVRDC tomato lines against Tomato yellow leaf curl virus, in collaboration with the Hebrew University, Israel (2003)

Research on effectiveness and feasibility of using botanicals in soil fertility and pest management in organic vegetable production (2003-on going)

International Foundation for Science Scholar (C/1997-1) from 1992-95: during this period, research on Passion fruit collar rot disease and identification of the pathogen and options for collar rot management. Technical backstopping and co-authorship of Prof. Patrick Van Damme

Consultancies/ project coordination

Consultant COLEACP/PIP, export agriculture IPM project in Uganda.No.0454/GG/Ssekyewa/01, and Lot No 0443/CC/PSM (01-10), 2005-2006

Technical adviser in the production of chilli, variety bird's eye with the welfare directorate of the Ugandan Army and with the USAID/APDF supported chilli export project, Joseph Matovu and Company Ltd/USAID-OCAP Grant, at Kibaale Resettlement Scheme, 2 months during 1996.

Worked as the country project coordinator for the GTZ-IPM Horticulture regional project, from 1994 to July 2000. As linkage to that project, also was coordination of the Institute for Insect Physiology and Ecology (ICIPE)/IFAD-funded project on Farmer Field Schools (1998-1999) and organisation of a regional Integrated Pest Management (IPM) training workshop, 8th to 18th June 1997, Kampala, Uganda.

In 1999, with assistance from Investment in Developing Export Agriculture (IDEA) project of USAID, I initiated production of virus free passion fruit planting material.

Around 1998 to 2000, co-moderation AFRIK-IPM listserv, an IPM electronic communication system with the main server based at Virginia Tech University, USA; and Chairperson of the Interim Steering Committee for establishment of a Horticulture Research and Development Network for the Association for the Strengthening of Agriculture Research in Eastern, Southern and Central Africa (ASARECA).

Since 2000 to 2004, technical adviser and coordinator for Seeds for Africa Charitable Trust/ Uganda Martyrs University Vegetable Gardening Projects in 157 Primary Schools, which aims at enhancing environment friendly agriculture education in primary schools. During this project, environment friendly vegetable gardens at schools were initiated, lead teachers educated and also educational materials and tools given out. Supported by the British museum, teachers and children were educated about the use of medicinal plants for pest management in agriculture and some plots of medicinal plants were established.

Founder Head of the Faculty of Agriculture, which was started in the year 2000, at Uganda Martyrs University, and development of hands-on curriculum for the Associate Bachelor's Degree and a Distance Learning Bachelor's Degrees in Agriculture, and in Organic Agriculture. Under this responsibility, an eco-tourism center for the University has been established with support from the Dutch–NMCP Netherlands organization expert. The site has trees and animal species identified and their uses documented based on existing indigenous knowledge.

2-Publications

Scientific Articles

a) International Journals

Shih S.L, +Green S.K, +Tsai W.S, *Ssekyewa C. 2005 Molecular Characterization of a New Tomato Begomovirus from Uganda. Plant Dis. 90:246

Ssekyewa, C., Opio, F.A., Swinburne, T.R., Van Damme, P.L., and Abubakar, Z.M. 1999 Sustainable management of collar rot disease of passion fruits in Uganda. International Journal of Pest Management, 1999, 45 (3) 173 – 177.

Ssekyewa, C., Swinburne, T.R., Van Damme, P.L., and Abubakar, Z.M., 1999 Passion fruit collar rot

disease occurrence in major growing districts of Uganda. Fruits, 1999, Volume 54, pp 405-411.

Submitted Articles

VanDamme P. L. and Ssekyewa C. 2006. A comparison of Tomato Leaf Curl Viruses Identified in Uganda with other Tomato Leaf Curl Viruses Identified Elsewhere in the World.
Journal of Phytopathology.

VanDamme P. L. and Ssekyewa C. 2006. Identification of Deoxyribonucleic Acid Tomato Viruses in Uganda. Journal of Phytopathology.

Ssekyewa C. and VanDamme P. L. J. 2006. Identification of Ribonucleic Acid Tomato Viruses in Uganda. Plant Disease (Journal)

Ssekyewa C. and VanDamme P. L. J. 2006. Relationship between tomato yellow leaf curl viruses and Bemisia tabaci whitefly vector in Uganda. International Journal of Pest Management

Conference proceedings

Ssekyewa, C., Nono-Womdim, R. and Kyamanywa, S. 2000 Incidence, distribution and characteristics of major tomato viruses in Uganda. Survey results. IPM -CRSP Workshop, 2001, Department of Crop Science, Makerere University.

Akemo, M.C., Ssekyewa, C., Bahindura, J. 1997 Evaluation of short day onion cultivars in Uganda. In Rabinowitch, H.D., Kimani, P.M., Peters, R. 1997 Proceedings of the First Eastern Africa Regional Alliums Workshop, 21 – 22 September 1994, KARI, Nairobi, Kenya. Xvii + 131pp. The Center for International Agricultural Development Cooperation (CINADCO), P.O.Box 7011, Tel.Aviv, Israel

Namirembe Sonko, R., Ssekyewa, C., and Akemo, M.C. 1997 The status of onion production and research in Uganda and prospects for future work. In Rabinowitch, H.D., Kimani, P.M., Peters, R. 1997 Proceedings of the First Eastern Africa Regional Alliums Workshop, 21 – 22 September 1994, KARI, Nairobi, Kenya. Xvii + 131pp. The Center for International Agricultural Development Cooperation (CINADCO), P.O.Box 7011, Tel.Aviv, Israel

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MEMBERSHIP

Board member for the International Society for Organic Agriculture Research (ISOFAR).
I am the Chairman of the Parents and Teachers Association of St.Joseph Nyenga Seminary, Uganda.

Founder member of Bio-Net International, which has the Taxonomy mandate for the fulfillment of the Convention for Biological Diversity.

Member of the Uganda Integrated Pest Management network

LANGUAGES

My mother language is Luganda. I speak and write good English. I do speak Swahili and know very few words of French and few words of Dutch. I have interest in learning more languages.

INTERESTS

Agro-ecotourism is one of my major interests and I am happy to see that through this activity it is possible for me to promote agriculture, and organic agriculture in particular, but in balance with my scientific understanding.

Lobbying and advocacy

Research, Education and development, especially for horticultural crops


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PVY