Chapter 1. Introduction

Tomato (*Solanum lycopersicum*) is one of the most widely grown vegetable crops with an annual world production of about 161 million metric tonnes valued at over 84.7 billion US dollars in 2012 (FAO, 2012). It is regarded as an important crop due to its high dietary value with respect to vitamin A and vitamin C (Campbell *et al.*, 2004; Jones *et al.*, 1997). Tomato is also gaining importance because it contains lycopene, a food component known to reduce the incidence of prostate cancer, heart and age-related diseases (Campbell *et al.*, 2004; Giovannucci, 1999). It is also an important source of income and employment and has the potential of value addition through processing. Low tomato yields have been reported due to a number of factors, of which pests and diseases have been ranked as major constraints to tomato production. In eastern and southern Africa, arthropods, fungal and bacterial diseases have been ranked as the major constraints to tomato production. Viral diseases have been rated as the third most important constraint. (Varela *et al.*, 2003).

Tomato spotted wilt virus (TSWV) is one of the most important plant viruses affecting vegetables and ornamental plants (Pappu *et al.*, 2009). It is among the ten most important viruses in the world (Scholthof *et al.*, 2011) and infects more than 1090 plant species in over 84 families. It is transmitted in a persistent propagative manner by at least nine thrips species (Pappu *et al.*, 2009; Riley *et al.*, 2011b; Whitfield *et al.*, 2005), where the virus is transmitted by the adult only when it is acquired by the larval stages (German *et al.*, 1992). Western flower thrips (*Frankliniella occidentalis*) has been reported as the most important vector due to their wide distribution, polyphagous nature and high fecundity (Riley *et al.*, 2011b). The virus is not seed transmitted or passed from adult to larvae during reproduction; therefore, a continuous supply of inoculum is required for infection to occur. Weed species have been shown to play an important role as reservoirs for the virus and its vectors between cropping

seasons (Gracia *et al.*, 1999; Kahn *et al.*, 2005; Okazaki *et al.*, 2009). Thus, understanding the dynamic of weed species in various production systems is important in effective management of the virus.

Several strategies have been developed for the management of TSWV and its vectors. Despite efforts in its management, TSWV has continued to cause serious losses worldwide due to its wide host range and presence of an efficient vector that is widely distributed and difficult to manage (Dianese *et al.*, 2011; Pappu *et al.*, 2009). Chemical control has been widely used in the management of TSWV but with limited success due to the high reproduction rate of the thrips and their development of resistance to insecticides (Dianese *et al.*, 2010; Gao *et al.*, 2012). This has been complicated by the migration of vectors from the infected fields, effective transmission after a short feeding time (Wijkamp *et al.*, 1995), resistance to chemicals and seclusive feeding behaviour of the vectors (Jensen, 2000; Persley *et al.*, 2006). Thrips species have also been reported to vary in their response to insecticides as well as in their efficiency in transmitting the virus (Jensen, 2000; Westmore *et al.*, 2013), hence documentation of the thrips species in crop production system is necessary to interpret thrips control data.

Use of resistant cultivars has been shown to be the best option for managing TSWV. Resistance has been identified in tomato, capsicum, peanut, and tobacco (Dianese *et al.*, 2010). In tomatoes, resistance has been attributed to the *Sw-5* gene, which resulted in significant reduction of losses due to TSWV in commercial tomato production, even under high thrips pressure (Aramburu *et al.*, 2010; Dianese *et al.*, 2010). Despite the occurrence of TSWV resistance breaking races which have been reported in Australia, South Africa, Europe and USA (Aramburu *et al.*, 2010; Latham & Jones, 1998; Thomas-Carroll & Jones, 2003;

Thompson & van Zijl, 1996), utilisation of resistant cultivars still remains one of the important methods in the management of TSWV.

The worldwide spread of *F. occidentalis* since the late 1970s contributed significantly to a resurgence of TSWV and its introduction to areas where it was not previously reported (Kirk & Terry, 2003). This led to several outbreaks of the disease arising from incursions by *F. Occidentalis* (Thompson & van Zijl, 1996). The disease has been introduced into several African countries (Dafalla, 2001; Sivparsad & Gubba, 2011; Ssekyewa, 2006; Wangai *et al.*, 2001); however, there is limited information on its distribution, host range and genetic diversity, except in South Africa. In Kenya, the disease was reported in 1999 (Wangai *et al.*, 2001), causing yield losses of up to 80% in tomatoes. Outbreaks of TSWV in tomato in Kenya have since been sporadic, often occurring for some years or seasons, leading to devastation and yield losses. Important vectors of TSWV, such as *F. occidentalis*, *Thrips tabaci* and *F. schultzei* have been reported in various vegetable production systems in Kenya (Kasina *et al.*, 2009; Nyasani *et al.*, 2010; Waiganjo *et al.*, 2008). However, information on the distribution and genetic diversity of TSWV and its vectors is still lacking. The current study was therefore aimed at establishing the distribution and genetic diversity of TSWV and its vectors, and establishing the role of weeds in disease epidemiology. It was also aimed at evaluating resistance as a management option in tomato production in Kenya.

The main goal of this study was to improve tomato productivity by establishing an effective management system for *Tomato spotted wilt virus* in tomato production in Kenya.

The specific objectives were:

1. To establish the occurrence, distribution and genetic diversity of TSWV after its incursion into Kenya.

2. To establish the diversity of thrips species and vectors of *Tomato spotted wilt virus* in tomato production systems in Kenya.

3. To determine the role of weeds in TSWV disease epidemiology in tomato production areas.

4. To establish the reaction of tomato cultivars grown in Kenya against TSWV and evaluate the presence of SW-5 gene using molecular techniques.

1.1 Thesis Outline

This thesis consists of a general introduction and literature review followed by four research chapters and concludes with a general discussion, conclusions and recommendations. Each of the research chapters has been prepared as an independent, publishable manuscript, except that figures and tables have been numbered to fit with the thesis format and the bibiliography have been combined. For this reason, on some occasions, there may be some repetition between chapters.

Chapter 2 contains the literature review. This provides relevant background information about the study, including tomato production and production constraints with focus on the importance of viruses, tospoviruses identity and detailed information about *Tomato spotted wilt virus,* including virus identity, vectors, transmission, detection and management.

Chapter 3 describes the occurrence, distribution and genetic diversity of TSWV and its vectors in major tomato production areas in Kenya. The chapter provides detailed information on farmer perspectives of the disease, distribution of TSWV in four major tomato production areas and its genetic diversity.

Chapter 4 describes thrips species occurring in tomato production in Kenya. This includes thrips species reported in tomato production and important vector species.

Chapter 5 describes the role of weeds in TSWV disease epidemiology in tomato production. This includes weeds occurring in tomato production, their susceptibility to TSWV, the ability to support thrips reproduction, and the transmission efficiency of TSWV from infected tomato to selected weed species identified in tomato production.

Chapter 6 describes the occurrence and utilisation of tomato cultivars resistant to TSWV. This includes the reaction of commercial tomato cultivars to TSWV infection and evaluation for the presence of the resistance gene.

Chapter 7 contains the general discussion, conclusions and recommendations of the research work and future research prospects.

Chapter 2. Literature Review

2.1 Introduction

Tomato (*Solanum lycopersicum*) is a member of the Solanaceae or nightshade family, which originated from South America and is now grown as a commercial vegetable crop around the world. This section highlights aspects of tomato production in Kenya and the major production constraints. The section also provides information on tospoviruses with specific focus on TSWV, its identification, vector species and management strategies for the virus and its vectors.

2.2 Tomato production in Kenya

Tomato is one of the most important vegetable crops widely grown in Kenya. It is mainly produced in the Central, Eastern, Rift Valley and Western provinces. Tomato is an important and an affordable source of vitamins to most households in Kenya. It also acts as a source of income for both small-scale and medium to large-scale commercial producers. Tomato production varies from small-scale farms, where it is mainly produced for local consumption to large-scale farms, where production is for extended fresh markets and processing. According to FAO reports, the annual production of tomatoes stood at 397,000 tonnes from 22,835 hectares in 2012and was valued at 236 million US dollars. This was lower than the 539,000 tonnes from 18,000 ha reported in 2010 (FAO, 2012). The major varieties grown in Kenya include Anna F1, Cal J, Chonto F1, Eden F1, Fortune Maker, Heinz 1350, Kilele F1, M82, Marglobe, Moneymaker, Nema 1400, Nouvelle, Onyx, Oxly Roma VF, Rio Grande, Super Marmade, Tylka F1 and Zawadi , (Masinde *et al.*, 2011; Varela *et al.*, 2003; Waiganjo *et al.*, 2006). Recently, greenhouse tomato production has been introduced, which allows production throughout the year.

Tomato is produced during the dry period, which has low to medium rainfall with supplementary irrigation (Masinde *et al.*, 2011). The crop has been found to grow well in warm conditions with optimum temperatures of 20°C – 25°C (Naika *et al.*, 2005; Srinivasan, 2010). Fruit setting and quality are affected by temperatures below 12°C or above 35°C (Srinivasan, 2010; Strange *et al.*, 2000). Low temperatures delay colour formation and ripening, while high temperatures inhibit fruit setting, lycopene development and flavour. Wet conditions have been associated with increased foliar disease infection and affect fruit ripening (Masinde *et al.*, 2011). The most desirable soils for tomato are deep, medium-textured sandy loams or loamy, fertile, well drained soils with high organic matter (Naika *et al.*, 2005).

2.3 Constraints to tomato production

The main constraints to tomato production in Kenya include lack of improved well-performing varieties, poor fruit setting because of heavy rains and excessively high temperatures, lack of inputs, sub-optimal crop husbandry and due to pests and diseases. A wide range of pests and diseases have been reported to affect tomatoes in Kenya. The major insect pests include western flower thrips (*Frankliniella occidentalis*), tobacco whitefly (*Bemisia tabaci*), African bollworm (*Helicoverpa armigera*), greenhouse whitefly (*Trialeurodes vaporariorum),* leaf miner (*Liriomyza sativae, L. trifolii* and *L. huidobrensis*), red spider mite (*Tetranychus urticae*), aphids (*Aphis gossypii*), and onion thrips (*Thrips tabaci*) (Nderitu *et al.*, 2010; Nyasani *et al.*, 2012; Varela *et al.*, 2003; Waiganjo *et al.*, 2008). The major diseases include bacterial wilt (*Ralstonia solanacearum*), late blight (*Phytophthora infestans*), bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*), bacterial canker of tomato (*Clavibacter michiganensis* subsp. *michiganensis*), early blight (*Alternaria solani*), Fusarium wilt (*Fusarium oxysporum* f.sp. *lycopersici*), viruses (*Tomato yellow leaf curl virus*

(TYLCV), *Tomato spotted wilt virus* (TSWV), *Tomato mosaic virus* (ToMV), *Tobacco mosaic virus* (TMV) and nematodes (*Meloidogyne incognita, M. hapla, M. javanica*) (Kimenju *et al.*, 2004; Otipa *et al.*, 2009; Varela *et al.*, 2003; Wangai *et al.*, 2001).

Viral diseases have been ranked as the third most important constraint in tomato production in Kenya due to the absence of enough scientific information on their importance. Tomato viruses have been reported to cause serious yield losses in various crops including tomato in many parts of the world (German *et al.*, 1992; Jones, 2005; Scholthof *et al.*, 2011). For most of the viruses transmitted by vectors, it has been shown that presence of vectors under favourable conditions can cause an epidemic. In Kenya, three tospoviruses namely TSWV on tomato, pepper and cucumber, *Iris yellow spot virus* (IYSV) on onion and leeks and *Tomato yellow ring virus* (TYRV) on tomato have been reported to occur (Birithia *et al.*, 2011; Birithia *et al.*, 2012; Ramkat *et al.*, 2008; Wangai *et al.*, 2001) and their main vectors *F. occidentalis, T. tabaci* and *F. schultzei* have also been reported in different horticultural systems (Kasina *et al.*, 2009; Nderitu *et al.*, 2010; Nyasani *et al.*, 2012; Waiganjo *et al.*, 2008). *Impatiens necrotic spot virus* (INSV) has been reported to occur in ornamental commercial production facilities but no further work has been undertaken. TSWV and TYLCV are among the most serious viruses leading to high yield losses in Kenyan tomato production with up to 80% of yield losses being reported due to TSWV infection (Wangai *et al.*, 2001).

2.4 Tospoviruses

Tospovirus constitutes the only genus with plant-infecting viruses in the family *Bunyaviridae,* while the other four genera (*Bunyavirus, Phlebovirus, Nairovirus* and *Hantavirus*) infect animals (Kormelink *et al.*, 2011; Silva *et al.*, 2001). Tospoviruses share many molecular characteristics with other viruses in this family which are also transmitted by different

arthropods (Jones, 2005; Kormelink *et al.*, 2011). The genus has some of the most important viruses infecting a wide range of plant species including vegetables, fruits and ornamental plants around the world (Gera *et al.*, 2000; Scholthof *et al.*, 2011). Tospoviruses are transmitted naturally by at least 14 thrips species consisting of eight *Frankliniella* species, three *Thrips* species, and one species of each *Scirtothrips, Dictyothrips* and *Ceratothripoides* (German *et al.*, 1992; Jones, 2005; Pappu *et al.*, 2009; Riley *et al.*, 2011b). The viruses are transmitted in a persistent, circulative and propagative manner whereby the virus is acquired by the larval stage, replicates in the insect and is transmitted by the adult (German *et al.*, 1992; Riley *et al.*, 2011b; Whitfield *et al.*, 2005; Wijkamp *et al.*, 1993).

The genus *Tospovirus* was established in 1992 based on molecular biology data, with *Tomato spotted wilt virus* as the sole species (German *et al.*, 1992). However, with extensive biological and molecular studies it has been revealed that the genus is composed of a number of species with distinct virulence profiles (de Avila *et al.*, 1993; Jones, 2005; Whitfield *et al.*, 2005). *Impatiens necrotic spot virus* (INSV), previously known as TSWV-1, was the second member of the genus *Tospovirus* to be identified, based on serological differences from TSWV (German *et al.*, 1992). Other tospoviruses serologically distinct from TSWV and INSV that were identified later include *Watermelon spot mosaic virus* (WSMoV) [TSWV-W], *Groundnut bud necrotic virus* (GBNV) and *Peanut yellow spot virus* (PYSV) (German *et al.*, 1992). Basically, identified tospoviruses have been classified to species level based on the morphology of the virion, genome structure and organisation, vector specificity, host range, and serological and molecular relationships (de Avila *et al.*, 1993; McMichael *et al.*, 2002; Persley *et al.*, 2006; Seepiban *et al.*, 2011).

The genus *Tospovirus* was initially subdivided into 10 serogroups based on serological cross reactions of the coat protein of the virus particle (Table 2.1) (Chen *et al.*, 2012; Jones, 2005;

Lin *et al.*, 2005). However, this system has been reported to be inappropriate due to the increase in the number of tospoviruses and cross reaction of the N-protein with members of different *Tospovirus* species (Chu *et al.*, 2001; Jones, 2005). This has led to the classification of the virus being based on serologically related groups where viruses in the same group are related. Two major groups have been identified tomato spotted wilt group (TSW), and watermelon silver mottle group (WSM) (Chu *et al.*, 2001; Lin *et al.*, 2005), while *Iris yellow spot virus* group with *Tomato yellow ring spot virus* (TYRV), Iris yellow spot virus (IYSV) and *Polygonum ringspot virus* (PolRSV) with similar serological reaction has been proposed as the third serogroup (Chen *et al.*, 2010; Ciuffo *et al.*, 2008). *Capsicum chlorosis virus* (CaCV) and *Calla lily spot virus* (CCSV) are additional species in the WSMo group (Chen *et al.*, 2010). *Impatiens necrotic spot virus* (INSV), *Groundnut yellow spot virus* (GYSV), *Groundnut chlorotic fan-spot virus* (GCFV) and *Melon yellow spot virus* (MYSV) have no clear serological relationship with other tospoviruses and are classified as distinct monotypes (Chu *et al.*, 2001; Lin *et al.*, 2005). *Physalis severe mottle virus* (PhySMV) has been reported to be identical with *Melon yellow spot virus* (MYSV) and thus they are considered to be the same species (Lin *et al.*, 2005). Identification of new species has been based on similarities in amino acid sequences of the N protein. Individuals with more than 90% identity are considered to belong to the same species, while those with less than 90% identity are considered to belong to distinct species (Chen *et al.*, 2012; Jones, 2005; Seepiban *et al.*, 2011).

Table 2.1 Classification of tospoviruses

Tospovirus*	**Serogroup**	**Group[a]**	**Nucleotide identities[b]**	**Derived amino acid identities[c]**
Tomato spotted wilt virus (TSWV)	I	TSW	100.0	100.0
Groundnut ringspot virus (GRSV)	II	TSW	76.7	78.3
Tomato chlorotic spot virus TCSV)	II	TSW	75.8	76.7
Impatiens necrotic spot virus (INSV)	III	-	57.0	55.3
Groundnut bud necrosis virus GBNV)	IV	WSMo	46.2	32.8
Watermelon bud necrosis virus (WBNV)	IV	WSMo	43.3	32.3
Watermelon silver mottle virus (WSMoV)	IV	WSMo	44.4	33.7
Groundnut yellow spot virus GYSV)	V	-	41.9	24.1
Iris yellow spot virus (IYSV)	VI	IYSV	47.1	37.3
Melon yellow spot virus (MYSV) =Physalis severe mottle Virus (PhySMV)	VII	WSMo	46.1	30.8
Chrysanthemum stem necrosis virus (CSNV)	VIII	TSW	74.0	76.0
Zucchini lethal chlorosis virus (ZLCV)	XI	TSW	72.7	72.9
Groundnut chlorotic fan-spot virus (GCFV)	X	-	43.6	24.4
Capsicum chlorotic virus (CaCV)	-	WSMo	41.6	28.9
Calla lily chlorotic spot virus (CCSV)	-	WSMo	39.6	23.4

[a] Serological group
[b, c] Percentage identity of the virus compared to TSWV
- Unclassified
Sourced: Jones, (2005) with additional information from Chen *et al*. (2010), (2012), Lin *et al*. (2005), and Seepiban *et al*. (2011)

TSWV is the type species of the genus *Tospovirus,* hence it is similar to most of the species in this genus. According to Chu *et al*. (2001), the TSWV group consists of GRSV, CSNV, TCSV and ZLCV (Table 2.1), which have been found to be closely related to TSWV. Viruses in this group have high identity based on their amino acid sequence. Garcia *et al*. (1999) reported that a virus disease in Argentina formerly believed to be caused by TSWV was found to be caused by a combination of three tospoviruses: GRSV, TCSV and TSWV. These three viruses produced indistinguishable field symptoms, infected the same crops and caused severe symptoms on vegetables and ornamentals. Similarly, primers prepared for identification of

TSWV were found to also amplify GRSV, TCSV and CSNV, indicating their close genetic similarity to TSWV (Boonham *et al.*, 2002).

The genus *Tospovirus* has a high numbers of virus species being discovered based on the amino acid sequences of the N nucleocapsid protein, serology, vector specificity and host range (Chen *et al.*, 2010; Pappu *et al.*, 2009; Zhou *et al.*, 2011). The genus represents a rapidly emerging virus pathogens with new species appearing regularly in recent times. So far there are at least 29 (11 formal and 18 tentative) *Tospovirus* species that have been identified, of which 11 species were recognised by the International Committee on Taxonomy of Viruses (ICTV) by 2014 (ICTV, 2014) (Table 2.2). Tospoviruses have been reported to be widely distributed in temperate, sub-tropical and tropical climatic conditions. The greatest number of the species have been reported in Asia and South America, of which 17 tospoviruses occur in Asia and at least 10 in South America (Table 2.2) (Chen *et al.*, 2012; Chiemsombat *et al.*, 2008; Jones, 2005; McMichael *et al.*, 2002; Pappu *et al.*, 2009; Persley *et al.*, 2006). The genetic diversity of *Tospovirus* species in Brazil has been shown to be one of the highest suggesting that South America is likely to be one of the centres of origin of this genus (Giordano *et al.*, 2010). However, only a few tospoviruses (GRSV, INSV, TSWV, IYSV and TYRV) have been reported in Africa, mainly in South Africa, Kenya and Uganda (Birithia *et al.*, 2011; Birithia *et al.*, 2012; du Toit *et al.*, 2007; Pappu *et al.*, 2009; Sivparsad & Gubba, 2008; Wangai *et al.*, 2001). The low number of tospoviruses recorded in Africa could be attributed to the lack of intensive surveys and research compared to those already conducted in South America and Asia (Pappu *et al.*, 2009).

Tospoviruses infect a wide range of host plants which include vegetables, legumes and ornamentals, as well as weeds and some native plants. The virus species in this genus infects

different host plants where some have a wide host range such as TSWV which infects over 1000 plant species and INSV which infects at least 300 plant species, while some others have limited host plants such as CaCV (5 plant species), and a single host for GCFV and ZLCV (Gracia *et al.*, 1999; Parrella *et al.*, 2003; Persley *et al.*, 2006). Consequently, new hosts of the virus species are being identified with more research being undertaken. Among the identified hosts of tospoviruses, weed species have been shown to play an important role as reservoirs for viruses when the main hosts are absent in the environment (Gracia *et al.*, 1999; Jones, 2005; Parrella *et al.*, 2003). Tospoviruses have been reported to produce varied symptoms that are associated with differences in the virus, host plants, cultivars, time of the year and environmental conditions (German *et al.*, 1992).

Table 2.2 A summary of geographical distribution, host range and vector species of the recognised and tentative tospovirus species

Tospovirus species and acronyms	**Geographical distribution**	**Hosts**	**Vector species**
Species recognised by 2014*			
Groundnut (*peanut*) *bud necrosis virus* (GBNV)	India, South-eastern Asia	Peanut (groundnut), tomato, capsicum, other grain legumes, weed species	*Thrips palmi, Frankliniella schultzei, Scirtothrips dorsalis*
Groundnut (*peanut*) *yellow spot virus* (GYSV)	India, Thailand	Peanut	*Scirtothrips dorsalis*
Groundnut ringspot virus (GRSV)	Brazil, Argentina, South Africa, USA	Peanut (groundnut), tomato, potato, lettuce, soybeans, weeds, ornamentals	*Frankliniella occidentalis*, *F. schultzei, F. intonsa, F. gemina*
Impatiens necrotic spot virus (INSV)	USA, West and South Europe, New Zealand, Japan, Egypt, Israel	Ornamentals, peanut, capsicum, potato, weed species	*F. occidentalis, F. fusca*, *F. intonsa*
Iris yellow spot virus (IYSV)	Australia, Brazil, Israel, Japan, Kenya, Netherlands, Uganda, USA	Iris, leek, onion	*T. tabaci, F. schultzei*
Polygonum ringspot virus (PolRSV)	Italy	*Polygonum convolvulus* L. and *Polygonum dumetorum* L.	*Dictyothrips betae*
Tomato chlorotic spot virus (TCSV)	Argentina, Brazil	Tomato, celery, lettuce, potato, sweet pepper, ornamentals, weeds	*F. occidentalis*, *F. intonsa*, *F. schultzei*
Tomato spotted wilt virus (TSWV)	Worldwide	Many hosts among crop, weed and ornamental species	*F. occidentalis*, *F schultzei, F. bispinosa*, *F. fusca*, *F. intonsa*, *T. tabaci, T. setosus, F. cephalica, F. gemina*
Watermelon bud necrosis virus (WBNV)	India	Watermelon	*T. palmi*
Watermelon silver mottle virus (WSMoV)	Japan, Taiwan	Watermelon, other cucurbits, tomato	*T. palmi*
Zucchini lethal chlorotic virus (ZLCV)	Brazil	Zucchini (*Cucurbita pepo*)	*F. zucchini*
Tentative and other species			
Alstroemeria nectrotic streak virus (ANSV)	Colombia	Alstoemelia	*F occidentalis*
Bean necrotic mosaic virus (BeNMV)	Brazil	Beans	?
Calla lily chlorotic spot virus (CCSV)	Taiwan	Calla lilies (*Zantedeschia* spp.)	*T. palmi*
Capsicum chlorosis virus (CaCV)	Australia, Taiwan, Thailand	Capsicum, tomato, peanut, *Hoya australis*, gloxinia	*T. palmi*, *Ceratothripoides claratris, F. schultzei*
Chrysanthemum stem necrosis virus (CNSV)	Brazil	Chrysanthemum	*F. occidentalis*, *F. schultzei*

Table 2. Continued

Tospovirus species and acronyms	Geographical distribution	Hosts	Vector species
Tentative and other species continued			
Groundnut (peanut) chlorotic fan-spot virus (GCFV)	Taiwan	Peanut	*S. dorsalis*
Gloxinia ringspot virus (GloxRSV)		Gloxinia	*?*
Hippeastrum chlorotic ringspot virus (HCRV)	China	Tobacco, tomato and numerous ornamental plants	?
Lisianthus necrotic ringspot virus (LNRV)	Japan	Lisianthus (*Eustoma grandiflorum*)	?
Melon severe mosaic virus (MeSMV)	Colombia, Mexico	Melon	*?*
Melon yellow spot virus (MYSV)**	Taiwan, Japan	Melon	*T. palmi*
Pepper chlorotic spot virus (PCSV)	Taiwan	Sweet pepper	?
Pepper necrotic spot virus (PNSV)	Peru	Pepper and tomato	?
Soybean vein necrosis associated virus (SVNaV)	USA	Soybean	*?*
Tomato necrosis virus (TNeV)	Thailand	Tomato	
Tomato necrotic ringspot virus (TNRV)	Thailand	Tomato, pepper, groundnut	*T. palmi, C. claratris*
Tomato yellow fruit, ring virus (TFYRV) (Syn: Tomato yellow ring virus) (TYRV)	Iran, Kenya	Tomato	*T. tabaci*
Tomato zonate spot virus (TZSV)	China	Tomato	*?*

? = Vector not yet known
* Represent species that have been listed by International Committee on Taxonomy of Viruses (ICTV) 2014 (ICTV, 2014)
** An isolate of MYSV has been characterised and reported in literature as Physalis severe mottle virus (PSMV)

Source: Persley *et al.* (2006); with additional information from Birithia *et al.* (2011); Cheng *et al.* (2014); Ciuffo *et al.* (2009); Ciuffo *et al.* (2010); Ciuffo *et al.* (2008); de Oliveira *et al.* (2012); Dong *et al.* (2008); Hassani-Mehraban *et al.* (2010); Hassani-Mehraban *et al.* (2011); Ohnishi *et al.* (2006); Pappu *et al.* (2009); Riley *et al.* (2011b); Seepiban *et al.* (2011); Shimomoto *et al.* (2014); Srinivasan *et al.* (2012); Torres *et al.* (2012); Xu *et al.* (2014); Zhou *et al.* (2011)

2.5 *Tomato spotted wilt virus* (TSWV)

2.5.1 History and economic importance of TSWV

Tomato spotted wilt virus was first reported in Australia in 1915 by Brittlebank (1919) and officially named *Tomato spotted wilt virus* by Samuel *et al.* (1930). The disease was confirmed to be vectored by thrips in the 1920s. In Africa, the earliest occurrence of the disease was the serious wilt disease reported in South Africa in 1905 (Thompson & van Zijl, 1996). The disease was later reported in West Cape, the Free State and the whole of Transvaal by 1939. TSWV has since been reported infecting horticultural crops, ornamentals and weeds in Africa, Asia, Australia, Europe, South America, USA, and New Zealand (EPPO, 2014; German *et al.*, 1992; Gracia *et al.*, 1999; Johnson *et al.*, 1995; Latham & Jones, 1997; Mumford *et al.*, 1996; Ochoa Martinez *et al.*, 1999; Sakimura, 1969; Thompson & van Zijl, 1996; Timmerman-Vaughan *et al.*, 2014; Wangai *et al.*, 2001; Westmore *et al.*, 2013; Wilson, 1998, 2001). The disease is widespread in South Africa and Reunion, and has restricted distribution in Kenya, Madagascar, Mauritius, Tunisia and Zimbabwe. It has been reported in other African countries, though no detail information have been provided (Fig. 2.1.) (EPPO, 2014; Thompson & van Zijl, 1996; Wangai *et al.*, 2001). Although the disease was reported more than a century ago in South Africa, there is still very limited information about the disease in other African countries. This could be attributed to lack of extensive surveys for tospoviruses in this region compared to other tropical and subtropical areas where high numbers of tospoviruses have been reported (Pappu *et al.*, 2009).

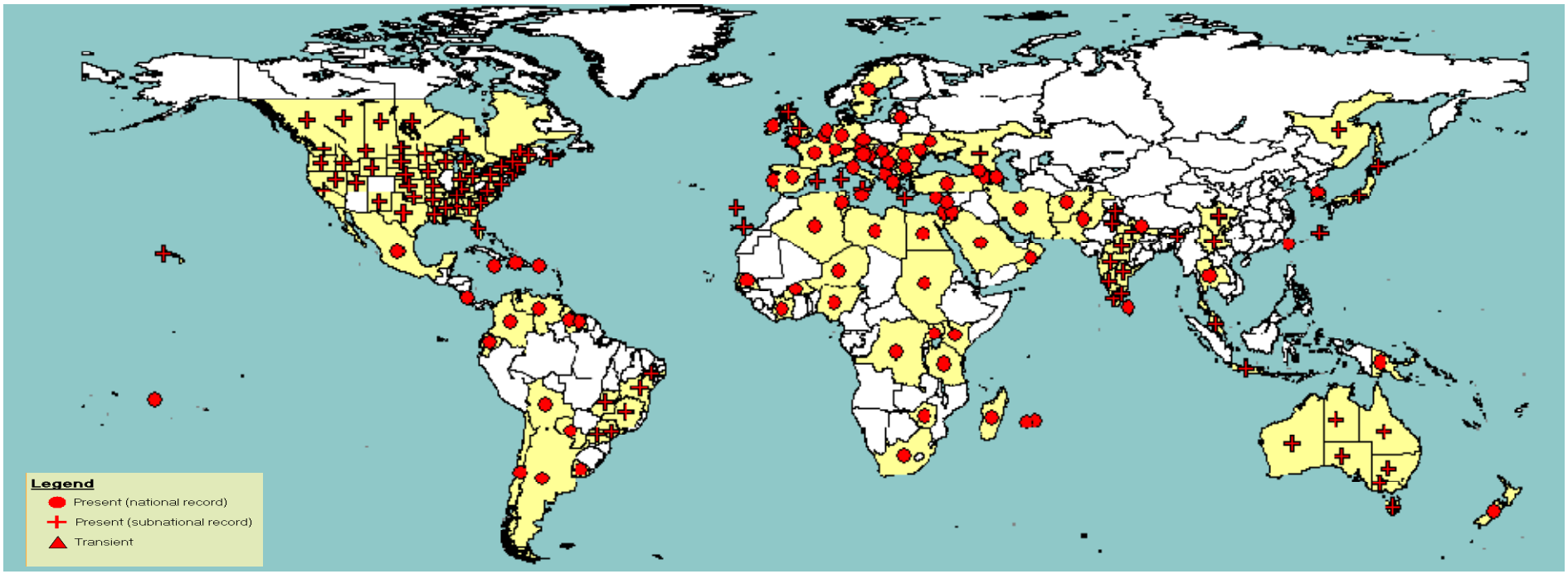

Figure 2.1 Worldwide distribution and status of *Tomato spotted wilt virus*

Source: PQR – Map generated from EPPO Plant Quarantine Data Retrieval system (EPPO, 2014).

TSWV has been rated among the most economically destructive and scientifically challenging plant viruses in the world (Scholthof *et al.*, 2011). This is due to its wide host range, wide distribution, and presence of an efficient vector species, *F. occidentalis* (Cho *et al.*, 1989; German *et al.*, 1992; Pappu *et al.*, 2009; Parrella *et al.*, 2003). The virus has been reported to cause huge economic losses in tomatoes, lettuce, tobacco and ornamental plants (Cho *et al.*, 1989; German *et al.*, 1992; Latham & Jones, 1997; Persley *et al.*, 2006). The epidemics are influenced by the availability of inoculum, the susceptibility of the host plant and the presence of competent thrips vectors (Jones, 2004; Pappu *et al.*, 2009; van de Wetering, 1999; Wijkamp *et al.*, 1995; Wijkamp *et al.*, 1993; Wilson, 1998, 2001). Environmental factors have also been shown to contribute to the successful establishment and spread of TSWV and its vectors even in areas previously reported to be unfavourable (Hanssen *et al.*, 2010). Despite extensive research on TSWV, it has continued to cause severe yield losses in tropical, sub-tropical and temperate regions (German *et al.*, 1992; Pappu *et al.*, 2009; Rosello *et al.*, 1996; Wijkamp *et al.*, 1995). The worldwide spread of *F. occidentalis*, which is the most efficient vector of TSWV through international trade, has led to renewed outbreaks of the disease and its introduction in areas where it was previously not known to occur (Kirk & Terry, 2003).

Significant yield losses resulting from TSWV infection has led to the loss of millions of dollars in crops such as tomato, potato, pepper, lettuce, tobacco and peanut in the USA (Hawaii, Georgia, North Carolina), Australia, and European countries (Cho *et al.*, 1989; Culbreath & Srinivasan, 2011; Groves *et al.*, 2002; Jones, 2005; Sevik & Arli-Sokmen, 2012; Tsompana *et al.*, 2005; Wilson, 2001). In Mexico, 90% yield losses were recorded on *Chrysanthemum* (Ochoa Martinez *et al.*, 1999), while production of *Begonia*, *Cyclamen* and *Impatiens* was found to be no longer economically viable due to their susceptibility to TSWV. In Hawaii, high yield losses enough to cease tomato production were recorded (Cho *et al.*,

1989), while in Brazil, Argentina, Spain, Portugal, Italy, USA and Australia it was rated as the most limiting factor in tomato production (Aramburu *et al.*, 2000; Gracia *et al.*, 1999; Latham & Jones, 1997; Moriones *et al.*, 1998; Pappu *et al.*, 2009; Persley *et al.*, 2006). Yield losses of up to 95% have been reported in susceptible cultivars where higher losses were observed when plants were infected early in the season (Pappu *et al.*, 2009; Riley *et al.*, 2011a; Saidi & Warade, 2008). TSWV has been reported to be a major constraint in tomato production in South Africa for last than two decades (Sivparsad & Gubba, 2011; Sivparsad & Gubba, 2008; Thompson & van Zijl, 1996). In Kenya, TSWV was first reported in 1999 and was reported cause yield losses of up to 80% in tomato production (Wangai *et al.*, 2001). Sporadic outbreaks of the disease have occurred in tomato production leading to noteworthy losses. However, it was observed that the greatest loss in tomato production was mainly due to production of unmarketable fruits rather than total yield reduction (Gordillo *et al.*, 2008; Ramkat *et al.*, 2006; Riley *et al.*, 2011a). Economic loss has also been due to additional management measures such as application of insecticide and rogueing of disease materials.

2.5.2 Genomic structure of TSWV

TSVW has pleomorphic, spherical particles 80–120 nm in diameter. The particle is enclosed in a lipid membrane with two glycoproteins (G_N and G_C) and encloses multiple copies of three nucleocapsids (Chiemsombat *et al.*, 2008; Chu *et al.*, 2001; Pappu *et al.*, 2009). Each nucleocapsid contains a single stranded RNA segment, and the RNA segments are designated as L (large), M (medium) and S (small) (Pappu, 2008; Whitfield *et al.*, 2005). The S RNA and M RNA are both ambisense, while the L RNA is of negative polarity (Chiemsombat *et al.*, 2008). The L RNA encodes for RNA dependent RNA polymerase (RdRp) which is responsible for virus replication and genome transcription (Fig 2.2) (de Haan *et al.*, 1990; Whitfield *et al.*, 2005). The M RNA segment encodes movement protein (NSm) for cell-to-cell transfer and the precursor for glycoproteins (G_N and G_C), involved in TSWV transmission

by thrips (German *et al.*, 1992; Sin *et al.*, 2005; Whitfield *et al.*, 2005). The S RNA segment encodes for non-structural protein (NSs), the gene silencing precursor that is responsible for counteracting the plant defence mechanism, and the nucleocapsid protein (NP) for encapsidation of the viral RNA (Pappu *et al.*, 2009; Whitfield *et al.*, 2005). The virion is composed of 5% nucleic acid (RNA), 70% protein, 5% carbohydrate and 20% lipid (Parrella *et al.*, 2003).

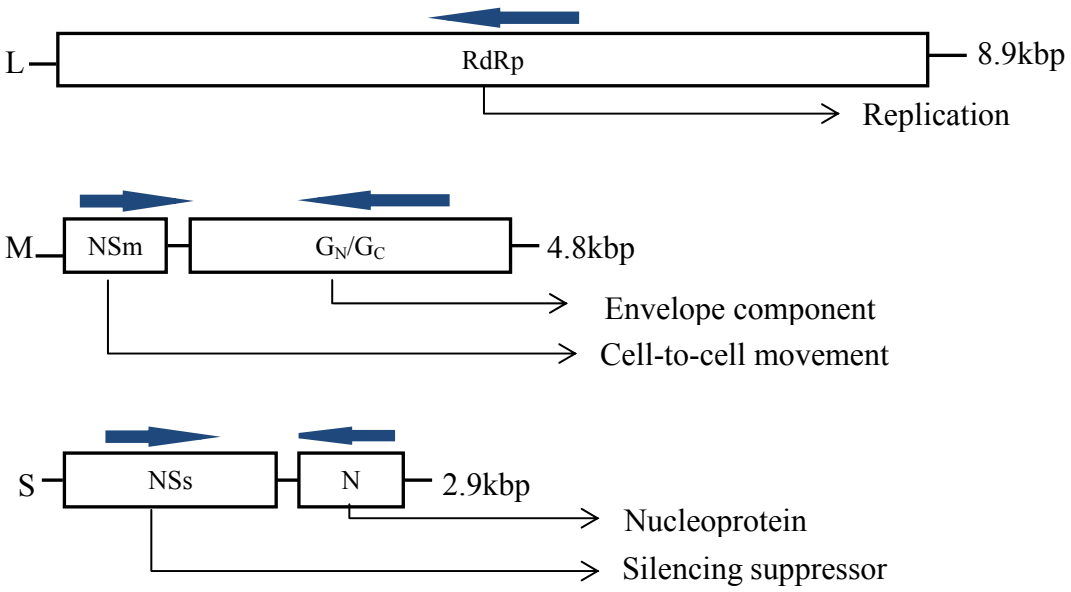

Figure 2.2 Genome organisation of *Tomato spotted wilt virus* and the functions of each gene (Pappu, 2008)

TSWV consists of a heterogeneous population with multiple strains which have been identified based on their characteristic symptoms on indicator plants, as well as on commercial host crops (Adkins, 2000; de Avila *et al.*, 1993). Molecular methods have also been used to more accurately determine strain differences (Naidu *et al.*, 2008; Tsompana *et al.*, 2005). TSWV isolates appear to vary greatly, depending on geographic source rather than

host crops, and due to high intraspecific-polymorphism and genetic differentiation between sub-populations (Hagen *et al.*, 2011; Tentchev *et al.*, 2011). Infection of plants with multiple strains leads to genomic reassortment which creates new variants, sometimes with increased virulence, or the ability of the strain to overcome the host's resistant genes (Aramburu *et al.*, 2010; Tsompana *et al.*, 2005). However, genome reassortment may occasionally reduce the level of virulence or the ability of vector thrips to transmit the virus (Nagata *et al.*, 2002; Naidu *et al.*, 2008; Sin *et al.*, 2005).

2.5.3 Host range of TSWV

Tomato spotted wilt virus has one of the widest host range of all know plant viruses infecting over 1,000 individual plant species most of which belong to the family Asteraceae (247 species), Solanaceae (172 species) and Fabaceae (60 species) (Parrella *et al.*, 2003). The virus infects one of the most diverse ranges of host plants compared to other plant-infecting viruses. Other tospoviruses have much narrower host ranges, and thus the broad host range of TSWV is not a characteristic of this genus (Jones, 2005). The virus infects important horticultural crops including tomato, sweet pepper, broad beans, common beans, potato, aubergine, tobacco, groundnut and lettuce (Pappu *et al.*, 2009; Parrella *et al.*, 2003). The virus has been recorded in a wide range of ornamental plants that include *Alstroemeria, Anemone, Antirrhinum, Araceae, Aster, Begonia, Bouvardia Calceolaria, Callistephus, Celosia, Cestrum, Columnea, Cyclamen, Dahlia, Dendranthema x grandiflorum, Eustoma, Fatsia japonica, Gazania, Gerbera, Gladiolus, Hydrangea, Impatiens, Iris, Kalanchoe, Leucanthemum, Limonium, Pelargonium, Ranunculus, Saintpaulia, Senecio cruentus, Sinningia, Tagetes, Verbena, Vinca* and *Zinnia* (Gracia *et al.*, 1999; Groves *et al.*, 2002; Ochoa Martinez *et al.*, 1999; Parrella *et al.*, 2003; Persley *et al.*, 2006). Many weed species are also hosts of the virus and act as important reservoirs for TSWV inoculum (Atakan *et al.*,

2013; Chatzivassiliou *et al.*, 2007; Cho *et al.*, 1987; Ochoa Martinez *et al.*, 1999; Parrella *et al.*, 2003; Wilson, 1998).

2.5.4 Symptoms of TSWV infections

Tomato spotted wilt virus produces highly variable symptoms which vary across plant species (German *et al.*, 1992; Persley *et al.*, 2006; Rosello *et al.*, 1996). The symptoms are influenced significantly by environmental factors, cultivars, nutritional status and plant age at the time of infection (Mandal *et al.*, 2008; Moriones *et al.*, 1998; Wilson, 2001). The susceptible host plants exhibit localised symptoms such as local chlorotic and necrotic lesions or systemic symptoms such as stunting, chlorotic ringspots, mottling, mosaic, wilting and necrotic areas (Cho *et al.*, 1989; Jones, 2005; Melzer *et al.*, 2012; Pappu *et al.*, 2009). Other symptoms including bronzing, purpling, curling, distortion, deformation, dark spots, die back stripes on petals, and necrosis have been observed (Jones, 2005).

In tomatoes, TSWV induce a wide range of symptoms including stunting, reddish brown rings on leaves which coalesce into necrotic leaf spots, mosaic, mottling, purpling, epinasty and fruit deformity, chlorotic ringspots on fruits and leaves and in severe cases, death of the plant (Aramburu *et al.*, 2000; Cho *et al.*, 1989; German *et al.*, 1992). Apart from causing severe fruit yield losses, TSWV infection results in blemished fruit with necrotic or chlorotic ringspots, which render the fruit unmarketable (Riley *et al.*, 2011b; Stevens *et al.*, 1995). Tomato fruits formed prior to infection appear normal, whereas fruits formed after infection may have pronounced symptoms ranging from pale green, yellow blotches and spots often with concentric zones of different colours or bumps on the surface (Jones, 2005; Sevik & Arli-Sokmen, 2012).

2.5.5 Identification of TSWV

Several biological, serological and molecular diagnostic methods have been developed for the identification of TSWV in plants and thrips species. These include use of indicator plants, enzyme linked immunosorbent assay (ELISA) using polyclonal antibodies (PAbs) and monoclonal antibodies (MAbs) (Sherwood *et al.*, 1989), dot blot hybridization (Rice *et al.*, 1990), reverse transcriptase–polymerase chain reaction (RT-PCR) (Mason *et al.*, 2003; Mumford *et al.*, 1996), immunocapture RT-PCR (IC-RT-PCR) (Wu *et al.*, 2009), real-time PCR (Debreczeni *et al.*, 2011; Mumford *et al.*, 2000; Roberts *et al.*, 2000), and electron microscopy (Milne, 1970). Procedures for detection of TSWV in thrips, which include ELISA, RT-PCR and real-time PCR have also been developed (Boonham *et al.*, 2002; Inoue *et al.*, 2004). Of these, ELISA and RT-PCR are the most widely used methods (Wu *et al.*, 2009).

Viruses in the genus *Tospovirus* have been shown to produce characteristic symptoms on different plants that are useful in diagnosis. Several indicator plants have been utilised in identification of TSWV which include *Petunia hybrida, Nicotiana glutinosa*, *N. benthamiana, Cucumis sativa, Chenopodium amaranticor, Chenopodium quinoa*, *Datura stramonium, N. rustica,* and *N. tabacum* (Table 2.3). *Petunia hybrida* is one of the most useful diagnostic species that has been shown to produce brown local lesions which develop, usually within 2–4 days after inoculation (Allen & Matteoni, 1991), whereas *Nicotiana glutinosa* and *N. benthamiana* develop large necrotic local lesions followed by a systemic mosaic and necrosis that sometimes produce severe symptoms on *N. benthamiana* (McMichael *et al.*, 2002; Parrella *et al.*, 2003). *Cucumis sativa* is also a reliable assay host as it develops chlorotic lesions on cotyledons 4–5 days after inoculation. Indicator plants that produce systemic symptoms such as *Datura stramonium, Nicotiana glutinosa, N. tabacum* and *Emilia*

sonchifolia have been utilised in the multiplication and maintenance of TSWV under greenhouse conditions (Chatzivassiliou *et al.*, 1999; Rotenberg *et al.*, 2009). They have also been used in biological characterisation of different tospoviruses, as each virus species produces different and characteristic symptoms.

Table 2.3 Symptoms produced by *Tomato spotted wilt virus* on some important indicator plants

Host	**Type of symptom**[a]	
	Local	**Systemic**
Amaranthaceae		
Gomphrena globosa cv. Buddy Purple	NR	NR, St
Asteraceae		
Latuca sativa	–	NS, MO
Chenopodiaceae		
Chenopodium quinoa	CS, NS,	Mo, W
Chenopodium amaranticolor	CS, NS	–
Fabaceae		
Arachis hypogaea	-	Mo, St, NS
Phaseolus vulgaris cv. Bountiful	NR, CS	-
Vigna unguiculata ssp. unguiculata cv. Black-Eye	NR	Mo, CS
Solanaceae		
Capsicum annuum cv. Yolo Wonder	CS	Mo, LD
Datura stramonium	NS	Mo, CN, LD, M, C, St, SN, W
Solanum lycopersicum cv. Grosse Lisse	–	NS, M, LD, St, C
Nicotiana benthamiana	CN	Mo, W
Nicotiana glutinosa	NR	Mo, LD, St, W
Nicotiana tabacum cv. Xanthi-nc	NR	–
Nicotiana rustica	NR	M, NR, LD
Petunia hydrida cv. Dreams White	NR	M

[a]Abbreviations: CN, chlorotic ringspots; CS, chlorotic spots; NR, necrotic ringspots; NS, necrotic spots; SN, severe necrosis; M, mosaic; Mo, mottle; C, chlorosis; LD, leaf deformation; St, stunting; W, wilt; –, no symptoms. Source: McMichael *et al.* (2002).

Electron microscopy has been used in the identification of TSWV based on the quasi-spherical envelope which is common only to tospoviruses. Leaf dip preparation, negative staining or thin sections of the infected plant tissue have been used for preparation of samples before identification (Milne, 1970; Sivparsad & Gubba, 2008). However, the use of the electron microscope for detection and diagnosis is restricted by the excessive time required and the small number of samples that can be examined.

Serological techniques (ImmunoStrips and ELISA) have been developed for the identification of TSWV and other tospoviruses mainly under field conditions. Several commercial kits have been developed and are widely used in TSWV identifications. The ImmunoStrips kits are easy to use, produce results within a short time can be used in the field with simple devices and can be operated by people with low skills. However, the main disadvantages of the kits are their high cost, low sensitivity and specificity. On the other hand, ELISA techniques are the most widely used for the identification of TSWV (Dang *et al.*, 2009; Wu *et al.*, 2009). Serological tests based on double antibody sandwich (DAS) ELISA, triple antibody sandwich (TAS) ELISA, tissue blot immunoassay (TBIA) and squash blot have been developed using polyclonal and monoclonal antibodies specific to TSWV (Chatzivassiliou *et al.*, 2000; Dang *et al.*, 2009; Whitfield *et al.*, 2003; Wu *et al.*, 2009). Furthermore, serological techniques have been developed for detection of TSWV in individual thrips (Cho *et al.*, 1988; Inoue *et al.*, 2004; Kresta *et al.*, 1995; Okazaki *et al.*, 2011). Although ELISA has been reported to be sensitive in the identification of TSWV (Dang *et al.*, 2009), the main limitation in the use of ELISA has been due to inaccuracy, including cross reactions with related tospoviruses.

Reverse transcriptase-polymerase chain reaction (RT-PCR) techniques for identification of tospoviruses including TSWV have been developed (Chen *et al.*, 2012; Kuwabara *et al.*, 2010; Mason *et al.*, 2003; Mumford *et al.*, 1996; Roberts *et al.*, 2000; Uga & Tsuda, 2005). This technique has been considered to be more accurate, sensitive and able to detect small amounts of viral RNA in infected plants (Mumford *et al.*, 1996; Roberts *et al.*, 2000). It has been used to detect multiple species of tospoviruses through multiplexing (Kuwabara *et al.*, 2010; Mumford *et al.*, 1996; Uga & Tsuda, 2005) or through the use of degenerate primers (Chen *et al.*, 2012; Kuwabara *et al.*, 2010; Uga & Tsuda, 2005). RT-PCR protocols for the detection of TSWV specifically in thrips have also been developed (Mason *et al.*, 2003).

A quantitative reverse transcriptase-PCR (qRT-PCR) protocol for the identification of TSWV in plants and thrips has also been developed based on TaqMan and SYBR green chemistries (Boonham *et al.*, 2002; Debreczeni *et al.*, 2011; Dietzgen *et al.*, 2005; Roberts *et al.*, 2000; Rotenberg *et al.*, 2009). The qRT-PCR methods have been shown to be more sensitive than conventional PCR and have the ability to quantify low concentrations of the virus in the plant (Mumford *et al.*, 2000; Roberts *et al.*, 2000). The internal controls included during analysis have been shown to increase the reliability of the assay (Boonham *et al.*, 2002). Quantitative RT-PCR diagnosis is useful in applications where high sensitivity, reliability, speed and quantitative data are required, such as seed indexing, identification of virus in reservoirs, field survey, screening of germplasm for resistance and disease forecasting (Dietzgen *et al.*, 2005; Roberts *et al.*, 2000). Furthermore, qRT-PCR has also been utilised to detect and quantify TSWV titre in individual thrips (Boonham *et al.*, 2002; Rotenberg *et al.*, 2009; Whitfield *et al.*, 2005), allowing early detection of TSWV in vectors leading to timely management of the virus.

2.6 Vectors of *Tomato spotted wilt virus*

Thrips (family *Thripidae*, order *Thysanoptera*) are the sole vectors of TSWV (Mound, 1997). TSWV is transmitted by at least nine species of thrips, including *F. occidentalis* (Nagata *et al.*, 2004; Wijkamp *et al.*, 1995), *F. schultzei* (Sakimura, 1969; Wijkamp *et al.*, 1995), *F. fusca* (Riley *et al.*, 2011b), *T. tabaci* (Wijkamp *et al.*, 1995), *T. setosus* (Tsuda *et al.*, 1996), *F. bispinosa* (Avila *et al.*, 2006), *F. intonsa* (Wijkamp *et al.*, 1995), *F. cephalica (Ohnishi et al., 2006)* and *F. gemina* (de Borbón *et al.*, 2006). Other thrips, including *Thrips palmi, Ceratothripoides claratris, Frankliniella zucchini, Dictyothrips betae* and *Scirtothrips dorsalis* have been reported to transmit other tospoviruses, but not TSWV (Riley *et al.*, 2011b). *F. occidentalis, F. schultzei, F. fusca* and *T. tabaci* are considered to be the most important vectors, but *F. occidentalis* has been shown to be the most efficient vector and like other vectors, readily retains the virus acquired during the larval stage throughout its life (Assis Filho *et al.*, 2004; Riley *et al.*, 2011b). Its high fecundity, efficient and rapid dispersal ability and polyphagous nature enable even a small number of the thrips to infect a substantial number of plants (German *et al.*, 1992; Riley *et al.*, 2011b).

2.6.1 *Frankliniella occidentalis* (western flower thrips)

Frankiliniella occidentalis (western flower thrips) is one of the most economically important pests affecting many horticultural crops. It is highly polyphagous affecting over 250 plant species in more than 62 families (EPPO, 2002). Apart from causing direct damage through oviposition and feeding, which leads to reduced aesthetic value due to feeding damage on foliage, flowers and fruits (Gao *et al.*, 2012), it has also been reported to be a vector of several tospoviruses, including TSWV, INSV, CSNV, GRSV and TCSV (Jones, 2005; Pappu *et al.*, 2009; Riley *et al.*, 2011b; Whitfield *et al.*, 2005; Wijkamp *et al.*, 1995).

Frankliniella occidentalis is reported to have originated from North America and until 1970 it was only known to occur in western Northern America and Mexico. It spread to the USA, Canada, the Netherlands and South Africa in the 1980s and later to other European countries in 1992, Japan in 1990, Australia in 1993 and South Korea in 1994 (Kirk & Terry, 2003). It has since been introduced to all the continents where it is reported to be an important pest of horticultural crops (Jones, 2005; Pappu *et al.*, 2009). In Kenya, it was introduced in 1989 and has since spread to horticultural production areas, where it has been reported as the main thrips species (Kedera & Kuria, 2003; Nyasani *et al.*, 2013).

F. occidentalis has a complete life cycle comprising the egg, two larval stages, two pupal stages and the adult. Eggs are laid singly in the plant tissue and hatch into the first larval instar, which start feeding immediately and later moult into the second larval instars (Mound, 1997). At the end of the second larval instar, it stops feeding and moves into the soil or plant debris and pupates into an immobile pupa that resembles the other stages but can be differentiated from the adult by its lack of wings. After the two pupal stages, the adult emerges from the pupa stage and starts feeding. Adults of *F. occidentalis* are usually yellowish to brown in colour, with distinctive tergal markings (brown bars) on a yellow-brown background, with darker forms also observed (Fig. 2.3). Rugman-Jones *et al.* (2010), using nuclear-mitochondrial barcoding, suggested that *F. occidentalis* may be a complex of two sympatric, cryptic species, also observing that darker specimens were more likely to be identified as one species than the other. Temperature has been reported to play an important role in the development of the thrips, where increased temperature significantly decreases the developmental time from egg to adult, with a mean developmental time approximately three times longer at 15^{o}C than at 32^{o}C (Kumm & Moritz, 2010; McDonald *et al.*, 1998; Olatinwo

et al., 2011). However, regardless of the temperature conditions, the larval stage has been shown to take the longest time (32%) before moulting to the pupa stage. The long duration in the larval stage has been reported to have an impact on virus acquisition and replication, leading to efficient transmission of the virus (Nagata *et al.*, 2002; Wijkamp *et al.*, 1995). Temperature has also been shown to affect the sex ratio, with a higher population of females observed at higher temperatures and a higher population of males observed at lower temperatures (Kumm & Moritz, 2010), which has a direct impact on the spread of TSWV. Thrips species are dispersed within a short distance through self-movement and over long distances through propagation materials and cut flowers during national and international trade (Huang *et al.*, 2010).

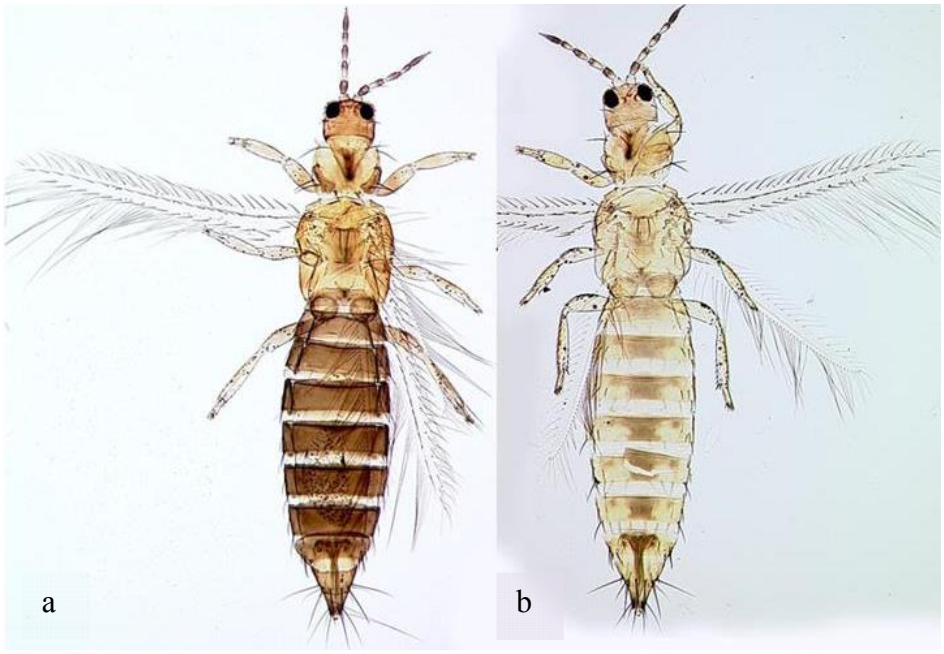

Figure 2.3 *Frankliniella occidentalis*, a) the dark form and b) the common pest form

Source: Mound (2006).

2.6.2 *Thrips tabaci*

Adults of *T. tabaci* are variable in colour, ranging from pale yellow, typically seen in warmer seasons, to brown in colder climates (Fig. 2.4). *T. tabaci* is a highly polyphagous thrips species that attacks plants in over 25 families and is recorded as an important pest of onion, garlic, cotton, tomato, wheat and ornamental plants (Mound, 2007). The species is widespread and has been reported in tropical and sub–tropical regions (Jones, 2005). *T. tabaci* is a vector of three tospoviruses: IYSV, TSWV and TYRV (Golnaraghi *et al.*, 2007; Srinivasan *et al.*, 2012; Wijkamp *et al.*, 1995). There has been conflicting information on the competence of *T. tabaci* to transmit TSWV (Cho *et al.*, 1988; Jacobson *et al.*, 2013; Westmore *et al.*, 2013; Wilson, 2001). Both poor and efficient transmitting populations have been observed in Europe, Australia and the United States (Chatzivassiliou *et al.*, 2000; Jacobson & Kennedy, 2013; Jenser *et al.*, 2003; Westmore *et al.*, 2013; Wilson, 2001). Although a group of *T. tabaci* belonging to the thelytokous group has been reported to be unable to transmit TSWV (Chatzivassiliou *et al.*, 2001; Chatzivassiliou *et al.*, 1999; Wijkamp *et al.*, 1995), Westmore *et al.* (2013) demonstrated the ability of the thelytokous population collected from potato to effectively transmit TSWV.

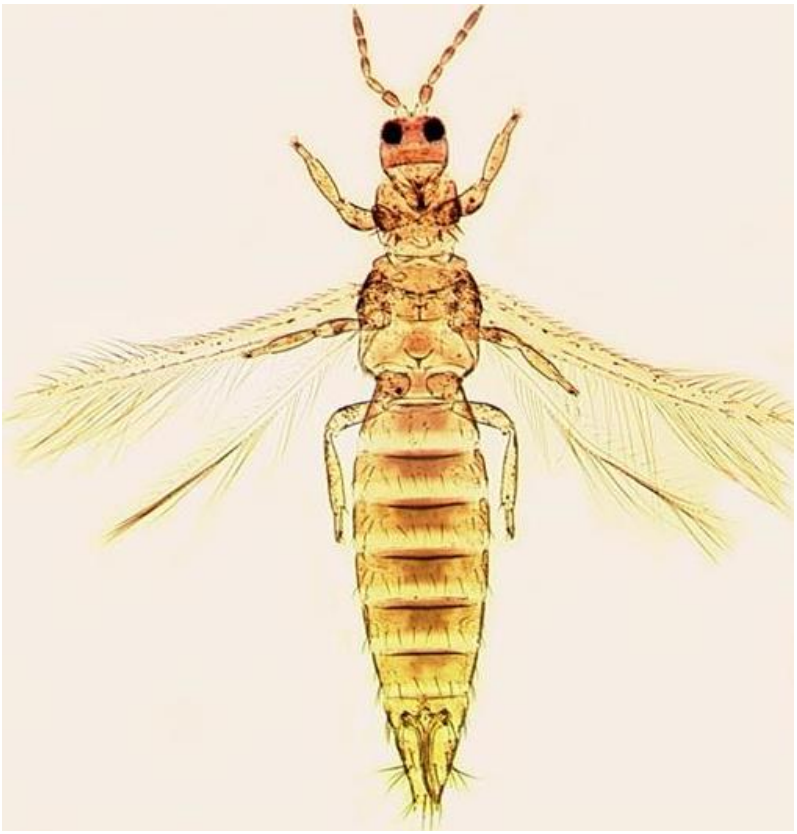

Figure 2.4 *Thrips tabaci*

Source: Mound (2007).

2.6.3 *Frankliniella schultzei*

Frankliniella schultzei occurs in two forms; pale and dark forms (Sakimura, 1969). The dark form is common in Australia, Africa and South America (Fig. 2.5), whereas the light (pale) form is common in parts of India and Northern Australia. However, both forms have been reported to occur in East Africa (Mound, 1996). *Frankliniella schultzei* is highly polyphagous and attacks plants in 83 species representing 35 families. The dark form has been reported to be more efficient in virus transmission than the lighter (pale) form (Wijkamp *et al.*, 1995). *F. schultzei* has been reported to transmit five tospoviruses, which include CSNV, GRSV,

GBNV, TCSV and TSWV (de Borbón *et al.*, 2006; Nagata *et al.*, 2004; Sakimura, 1969; Wijkamp *et al.*, 1995),

Figure 2.5 *Frankliniella schultzei* female of dark form.

Source: Tree (2010).

2.6.4 Identification of thrips

Thrips have largely been identified based on their morphological characteristics (Mound *et al.*, 2014; Riley *et al.*, 2011b). Their separation into genera is based on characters such as the number, size and location of the major setae on the head, pronotum, forewing and abdominal tergite II, as well as colour characteristics (Mound *et al.*, 2009). Diagnostic keys for the identification of thrips species, including *F. occidentalis, T. tabaci* and *F. schultzei* have been

developed based on morphological characteristics (Moritz *et al.*, 2013; Mound *et al.*, 2009; Mound *et al.*, 2014). However, identification of larval stages for different thrips species has not been possible with the use of the morphological identification keys (Huang *et al.*, 2010). High variation found within the thrips populations together with their minute size, scarcity of solid morphological features and need for expertise makes their identification more difficult (Brunner *et al.*, 2004; Kadirvel *et al.*, 2013). Furthermore, loss of taxonomic experts has spurred the need to find faster methods for species identification to replace or supplement the current methods which rely heavily on morphological–based procedures that are time consuming and occasionally do not provide the desired results (Packer *et al.*, 2009).

Molecular techniques have been identified as a quick, reliable and accurate method for identification of thrips species. They provide a powerful tool in the identification of species and investigation through phylogenetic relationships in insects (Hebert *et al.*, 2003; Kadirvel *et al.*, 2013). They have also contributed to an understanding of natural genetic diversity and speciation (Brunner *et al.*, 2004). DNA–based analyses designed to utilise specific primers for the identification of thrips species such as *F. occidentalis* and *T. palmi* have been developed, optimised and used for identification of both adults and larval stages (Huang *et al.*, 2010; Walsh *et al.*, 2005). However, use of DNA barcoding using the mitochondrial cytochrome oxidase 1 gene (mtCO1) has been shown to be simple, more accurate, applicable for screening of large samples and does not require taxonomic expertise (Hebert *et al.*, 2003; Herbert *et al.*, 2007; Kadirvel *et al.*, 2013). The protocol has been evaluated and found to be effective in the identification of different thrips species and genera that coexist in particular cropping systems (Kadirvel *et al.*, 2013; Karimi *et al.*, 2010; Timm *et al.*, 2008; Zhang *et al.*, 2012). For instance, based on mitochondrial cytochrome oxidase 1 (CO1) sequences, *T. tabaci* was classified into three groups consisting of the tobacco group, the arrhenotokous group and the

thelytokous group (Brunner *et al.*, 2004). Arrhenotokous and thelytokous groups are polyphagous and represent two reproductive forms: arrhenotokous, where parthenogenetically produced progeny are males; and thelytokous, where parthenogenetically produced progeny are females (Brunner *et al.*, 2004; Jacobson & Kennedy, 2013; Toda & Murai, 2007). Similar observations were made with *F. occidentalis,* where the species was divided into two genetically divergent groups that may represent cryptic species (Rugman-Jones *et al.*, 2010). Analysis of the CO1 gene has also suggested that insects identified morphologically as *F. schultzei* may belong to more than one species (Kadirvel *et al.*, 2013). These methods offer a quick and reliable identification of all stages of thrips and can be utililised in routine identification as well as in quarantine systems.

2.6.5 TSWV acquisition and transmission

Several studies have reviewed the interaction between TSWV and thrips (Assis Filho *et al.*, 2002; German *et al.*, 1992; Kritzman *et al.*, 2002; Rosello *et al.*, 1996; Ullman *et al.*, 1995). The transmission process is affected by virus-vector interaction, which involves acquisition, replication and movement within the insect and finally exit from the vector (Kritzman *et al.*, 2002; Ullman *et al.*, 1995; Whitfield *et al.*, 2008; Whitfield *et al.*, 2005). A unique thrips-tospovirus relationship has been observed where the adult is only able to transmit TSWV when it is acquired during the larval stages (Jones, 2005; Persley *et al.*, 2006; Whitfield *et al.*, 2005). Although adult thrips species are able to acquire the virus from TSWV–infected plants, it has been reported that they are not able to transmit the virus (Assis Filho *et al.*, 2004; Kritzman *et al.*, 2002). Once the virus is acquired by the larval instars, especially the first larval instar, it replicates in the insect before it is transmitted by adults and occasionally by the late stages of the second larval instar (Nagata *et al.*, 2002; Nakahara, 1997). It has been reported that the virus has to traverse at least six membrane barriers before transmission can

occur (Ullman *et al.*, 1989; Whitfield *et al.*, 2008). After acquisition, the virus enters the midgut lumen before entering the insect cell through a pH gradient and infecting the midgut epithelial cells (Johansen, 2002). The virus replicates and spreads to the adjacent midgut cells before moving into muscle cells surrounding the midgut. Finally, the virus infects the primary salivary gland and enters and accumulates in the salivary gland of the thrips (Assis Filho *et al.*, 2004; Assis Filho *et al.*, 2002; Kritzman *et al.*, 2002; Wijkamp *et al.*, 1993). The latent period of approximately three to seven days is required for the virus to replicate in the insect before it can be transmitted (Groves *et al.*, 2002). The replication of the virus in the midgut and subsequent migration from the midgut to the visceral muscle cells and salivary glands are crucial factors for vector competence (Johansen, 2002; Nagata *et al.*, 2002).

The vector-virus relationships where the virus should be acquired during the larval stage for infection to occur have implications in the continuous infection and spread of the virus. TSWV is not seed transmitted and there are no reports of transovarial transmission from the adult thrips to the offspring (German *et al.*, 1992; Pappu, 2008; Wijkamp *et al.*, 1995). This indicates therefore, that susceptible crops with overlapping production season, presence of susceptible weeds and thrips vectors play an important role in continuous infection and epidemics (Pappu, 2008; Pappu *et al.*, 2009). This is because thrips have to acquire TSWV at the larval stage for infection to occur in each of the generations (Pappu *et al.*, 2009; Riley *et al.*, 2011b).

The proportion of viruliferous individuals in a population is important in the transmission of the virus. The number of viruliferous thrips is determined by the number of first larval instars fed on TSWV-infected plants, the duration they are fed and virus concentration (Kritzman *et al.*, 2002; Whitfield *et al.*, 2005). German *et al.* (1992) observed that increased acquisition

time resulted in an increased number of viruliferous thrips. On the other hand, Rotenberg *et al*. (2009) found that the virus titre in an individual thrips affected the efficiency of transmission and the number of times viruliferous thrips can transmit TSWV. The incidence of viruliferous thrips has been shown to be an important factor contributing to TSWV outbreaks (Okazaki *et al.*, 2007). The amount of virus harboured by the thrips has epidemiological implications, as high virus titre leads to increased primary inoculum and thus high disease incidence. This is particularly important for viruliferous thrips migrating from outside the field, since they act as a primary source of inoculum for TSWV early in the production season (Rotenberg *et al.*, 2009). Thus, early prediction of viruliferous thrips population is an important part of epidemic risk prediction and their management (Okazaki *et al.*, 2011).

A unique interaction between TSWV and thrips has been reported which leads to an intimate association and mutualistic relationship (Assis Filho *et al.*, 2002; Kritzman *et al.*, 2002; Maris *et al.*, 2004; Sin *et al.*, 2005). TSWV infection has been shown to improve host suitability for its vectors, leading to increased preference in feeding and reproduction (Maris *et al.*, 2004). *F. occidentalis* females reportedly oviposited larger numbers of eggs on TSWV-infected host plants resulting in more viruliferous thrips and thereby increasing TSWV transmission (Maris *et al.*, 2004).

Although TSWV has been identified from all parts of plants, distribution is uneven within the plant and from plant to plant (Dietzgen *et al.*, 2005; Kresta *et al.*, 1995; Wilson, 2001). The concentration of the virus was reported to be unevenly distributed within individual peanut plants and in *Ranunculus* tubers (Kresta *et al.*, 1995; Whitfield *et al.*, 2003). It was further established that the virus concentration was higher in young, developing terminal tissue in

peanut and tomato plants (Dietzgen *et al.*, 2005; Kresta *et al.*, 1995; Wilson, 2001). Variation in virus distribution has implications for diagnosis of the virus and virus transmission. German *et al.* (1992) reported that variation in virus distribution had an effect on the efficiency of virus acquisition by thrips. Furthermore, the uneven virus distribution has an effect on the reliability of the diagnostic method, which implies that sampling of materials for analysis is critical.

2.7 Weeds as reservoirs of TSWV

Weeds play an important role as alternate hosts of tospoviruses, where they have been shown to act as reservoirs of the virus between cropping seasons (Gracia *et al.*, 1999; Parrella *et al.*, 2003). Susceptible weed species that remain in the field after harvesting, or that occurs near the production field, have been shown to act as a sink for the virus between cropping seasons (Chatzivassiliou *et al.*, 2001; Chatzivassiliou *et al.*, 2007). Gracia *et al.* (1999) reported that weed species serve as sources of inoculum between cropping seasons for TSWV, INSV, GRSV, GBNV and TCSV. The ability of weeds to grow fast, reproduce rapidly and withstand harsh conditions enables them play an important role in the epidemiology of tospoviruses.

TSWV infects a wide range of annual, biennial and perennial weeds under different climatic conditions (Groves *et al.*, 2002; Persley *et al.*, 2006). Perennial weeds have been shown to serve as persistent source of virus inoculum that is later passed on to annual weeds where it replicates before it is further spread to susceptible crops. *Arctotheca calendula* (cape weed) and *Sonchus asper* (sowthistle) were reported as weed species with the highest incidence of TSWV in Western Australia, while in Queensland, *Bidens pilosa* was frequently infected with TSWV and snake weed (*Stachytarpheta jamaicensis*), a perennial herb in pastures acted as a

virus survival host during hot summers when vegetables were rarely grown (Persley *et al.*, 2006; Wilson, 1998). On the other hand, summer and winter weed species acted as reservoirs for TSWV and hence served as a source of inoculum to crops in spring (Kahn *et al.*, 2005; Srinivasan *et al.*, 2014).

Weeds have also been shown to act as suitable feeding and reproductive hosts for thrips species. The ability of weed species to support thrips oviposition and reproduction has been attributed to the nutritional quality of the host plant and presence or absence of plant defence compounds (Koschier *et al.*, 2007; Scott Brown *et al.*, 2002). Nyasani *et al*. (2013) reported high feeding and oviposition of *F. occidentalis* on *Curcubita pepo* and French bean (*Phaseolus vurgaris*). *Galinsoga parviflora* was regarded as the most preferred feeding and oviposition host among the weeds commonly occurring in French bean fields (Nyasani *et al.*, 2013). Weed species that are susceptible to tospoviruses and support thrips reproduction are important in the introduction and spread of the virus in cropping systems (Groves *et al.*, 2002; Jenser *et al.*, 2003). They have been shown to act as reservoirs of the virus and support population build-up of viruliferous thrips, which may lead to a disease epidemic (Groves *et al.*, 2002). On the other hand, plants that are susceptible to the virus but do not support thrips reproduction have been considered not to contribute towards further virus spread (Duffus, 1971).

2.8 Management of TSWV and its vectors

2.8.1 Management of TSWV through control of vectors

Management of TSWV is mainly focused on the control of vectors, but their management has been reported to be extremely difficult due to their polyphagous nature, high fecundity,

adaptation capacity and resistance to insecticide (Jensen, 2000; Jones, 2005; Riley & Pappu, 2004). Although several management options have been developed, including control of alternate hosts, crop rotation, use of reflective mulches and use of thrips netting, their application has only been marginally effective (Jensen, 2000; Pappu *et al.*, 2009).

Cultural and agronomic practices have been applied to reduce virus infection and thrips populations. Since crops are most vulnerable to virus infection during the early stages of growth, protecting seedlings from infestation by thrips has been shown to reduce TSWV incidence (Jensen, 2000). Other cultural and agronomic practices that have been developed include use of non-host crops as opposed to leaving the fields fallow during rotation, removal of infected plants, management of weeds, avoiding high levels of nitrogen and intercropping with non-host crops (Jensen, 2000; Jones, 2005; Momol *et al.*, 2004). The use of UV reflective mulch to modify thrips behaviour, application of horticultural oils, plant extracts and film forming products, and use of netting in extensive protected cultivation to exclude thrips have also been used in the management of thrips (Jensen, 2000; Pappu *et al.*, 2009; Riley & Pappu, 2004).

Biological control strategies have been used in the management of thrips with varied levels of success. Use of predatory mites, *Typhlodromips montdorensis, Neoseiulus cucumeris* and *Hypoaspis miles,* for the control of *F. occidentalis* and *T. tabaci* in protected systems in Australia led to reduced thrips populations (Rahman *et al.*, 2011) whereas *Neoseiulus* (*Amblyseius*) *cucumeris* reduced WFT in French bean production in Kenya (Nyasani *et al.*, 2015). *Amblydromalus limonicus* has also been used in the control of WFT (Knapp *et al.*, 2013). Moreover, *Metarhizium anisopliae* and *Beauveria bassiana* were identified as effective in the control of thrips (Niassy *et al.*, 2012; Nyasani *et al.*, 2015). Other biological control

agents include *Verticillium lecanii,* several species of *Orius* and entomopathogenic nematodes. The main drawback in the use of biological control is that although they are effective in the reducing thrips populations over time, they have not been effective in preventing initial TSWV infections from thrips migrating from an external source.

Chemical control, which is one of the most important and widely used methods for the management of thrips, is often constrained by the seclusive behaviour of the thrips, where the eggs are laid inside plant tissue, pupation occurs in the soil and adults feed in protected areas such as flowers and buds (Herbert *et al.*, 2007; Jensen, 2000). Thrips' resistance to several groups or classes of insecticides, including organophosphates, carbamates, pyrethroids, abamectines and organochlorines have been reported (Brødsgaard, 1994; Gao *et al.*, 2012; Immaranju *et al.*, 1992; Momol *et al.*, 2004; Zhao *et al.*, 1995). Over 153 cases of resistance to insecticides by western flower thrips involving at least seven chemical classes have occurred throughout the world (Gao *et al.*, 2012). Use of chemical control has also been affected by the ban of some registered products because of their harmful effect on humans and the environment, and the lengthy process needed to discover, develop and register novel insecticides for commercial use (Gao *et al.*, 2012; Hillocks, 2012). However, judicious use of chemicals, which include rotation of different chemicals with different modes of action, has been reported to reduce the development of resistance among thrips species (Gao *et al.*, 2012).

Integrated pest management (IPM) has been used with some level of success (Riley & Pappu, 2004). Use of reflective mulch, host resistance and chemical insecticides were shown to reduce infestation from thrips species, thereby increasing tomato production (Riley & Pappu, 2004). Utilisation of non-chemical strategies such as biological control agents, cultural control, management of weeds and use of host resistance have been shown to reduce

population pressure (Gao *et al.*, 2012; Riley & Pappu, 2004). Furthermore, use of *Metahrizium anisopliae*, *N. cucumeris* and imidacloprid in combination significantly reduced WFT in French bean production, hence were found have potential in developing an integrated pest management program (Nyasani *et al.*, 2015). Although there is potential for the use of IPM in the management of thrips species, there is need for further work to establish its ability to reduce virus infection.

2.8.2 Resistant cultivars

Use of resistant cultivars, has been reported to be the best management option for TSWV (Gordillo *et al.*, 2008; Persley *et al.*, 2006; Riley *et al.*, 2011a). Resistance has been identified from wild as well as cultivated species, leading to development of resistant cultivars in tomato, capsicum, tobacco and peanut (Dianese *et al.*, 2010). In *Capsicum*, resistance was observed in *Capsicum chinensis* and *C. baccatum* carrying the single dominant gene *Tsw* (Cebolla-Cornejo *et al.*, 2003). Several resistant cultivars have been developed using this gene; however, the effectiveness of its resistance can be compromised by high temperature and when plants are inoculated at early stages (Sharman & Persley, 2006). On the other hand, resistances have been found in domesticated and wild *Solanum* species including *Solanum peruvianum*, *S. hirsutum*, *S. pimpinillefolium* and *S. lycopersicon* (Dianese *et al.*, 2010; Gordillo *et al.*, 2008). *Solanum peruvianum* and *S. pimpinillefolium* have been reported to be the best source of resistance (Dianese *et al.*, 2010; Gordillo *et al.*, 2008; Jones, 2005; Persley *et al.*, 2006; Stevens *et al.*, 1994).

Eight major genes conferring resistance against TSWV in tomato have been identified including five dominant ones namely *Sw-1*^{a}, *Sw-1*^{b}, *Sw-5, Sw-6* and *Sw-7,* and three recessive (*sw-2, sw-3, sw-4*) (Gordillo *et al.*, 2008; Stevens *et al.*, 1994). Among these, *Sw-5* has been

shown to be the most effective resistance gene with broad resistance (Saidi & Warade, 2008; Stevens *et al.*, 1994). The resistance mediated by *Sw-5* follows the gene-for-gene relationship by triggering the typical hypersensitive response around the TSWV infection site and limiting virus spread in the plant (Peiro *et al.*, 2014). The *Sw-5* gene is located on the telomeric region of the long arm of chromosome 9 (Brommonschenkel *et al.*, 2000). It has a highly conserved binding site and resides within a complex cluster comprising five linked paralogues denoted *Sw-5a* to *Sw-5e* (Spassova *et al.*, 2001). The *Sw-5b* is the only gene that confers resistance against TSWV. It was found that cultivars with the *Sw-5* gene had broad spectrum resistance with high stability and were free from systemic infection by TSWV isolates as well as other related tospoviruses such as GRSV and TCSV (Dianese *et al.*, 2010; Dianese *et al.*, 2011; Stevens *et al.*, 1994). Daniese *et al*. (2011) reported that the gene also showed resistance to CNSV. A significant positive impact has been reported, resulting in reduced losses from TSWV in commercial tomato production under high pressure of viruliferous thrips species (Aramburu *et al.*, 2010; Dianese *et al.*, 2010; Giordano *et al.*, 2000; Stevens *et al.*, 1994).

Despite the success in the use of resistant tomato cultivars, a low proportion of systemic infection has been observed in some resistant tomato cultivars carrying the *Sw-5* dominant gene due to resistance breaking strains of TSWV (Aramburu *et al.*, 2010). Resistance breaking races have been identified in Australia (Latham & Jones, 1998), Spain (Aramburu & Marti, 2003), South Africa (Thompson & van Zijl, 1996), Italy (Ciuffo *et al.*, 2005) and Hawaii (Cho *et al.*, 1996). The number of resistance breaking cases is increasing (Aramburu *et al.*, 2010) and this has led to further research to establish a more reliable resistant gene. Accordingly, *Sw-7* was identified as effective against the resistance breaking races and is being introgressed into commercial lines (Stevens *et al.*, 2006).

2.8.3 Thrips resistance

Plant host resistance has been used in the management of TSWV with success. However, occurrence of resistance breaking races has necessitated further research on alternative management options for TSWV. Thrips resistance has been identified as a viable alternative with where resistance have been reported in chrysanthemum, senecio, cucumber, leeks, pepper and tomatoes (Leiss *et al.*, 2009; Maris *et al.*, 2003a; Maris *et al.*, 2002; Mirnezhad *et al.*, 2010; van de Wetering, 1999). Resistance of plant species to thrips has been attributed to the production of metabolites affecting the feeding habit of the insects (Leiss *et al.*, 2009; Mirnezhad *et al.*, 2010). Maharijaya *et al.* (2011) reported reduction of *F. occidentalis* and *T. parvispinus* in thrips–resistant *Capsicum* plants. *Groundnut bud necrotic virus* (GBNV) infection was reduced in thrips–resistant groundnuts, while TSWV infection was delayed in thrips resistant pepper (Maris *et al.*, 2003a). However, virus spread was increased in thrips–resistant chrysanthemum, which was attributed to changes in thrips feeding behaviour (van de Wetering, 1999). Thrips populations have been shown to be an important factor in the spread of TSWV, hence alternative methods for their control are important in the overall management of the virus (Maris *et al.*, 2002).

Thrips-resistant tomato were found to produced metabolites such as acyl sugars, which were responsible for reducing thrips infestation (Mirnezhad *et al.*, 2010). Resistance to thrips has been observed in wild tomato *L. pennelii* and *L. hirsutum,* but *L. peruvianum* which is resistant to TSWV was found to be susceptible to thrips infestation (Dianese *et al.*, 2010; Mirnezhad *et al.*, 2010; Stevens *et al.*, 1994). Reaction of some tomato cultivars to thrips has been established, but their subsequent effect on the spread of TSWV is still unknown.

2.9 Molecular Marker Assisted Breeding

Molecular marker assisted selection (MAS) has been developed and used for identification and mapping of the genes responsible for numerous agriculturally important traits in tomato such as resistance or tolerance to biotic and abiotic stress, and fruit and flower–related attributes (Panthee & Foolad, 2011). MAS provides an opportunity for the selection of important traits based on genotype as opposed to the trait itself, hence overcoming the limitations associated with phenotypic selection (Panthee & Foolad, 2011). Detection can be done at any growth stage and offers a convenient selection plan to breeders. MAS has been widely used by both the private and public sectors in tomato breeding programs, especially for simple inherited traits resistant to disease (Foolad, 2007; Panthee & Foolad, 2011).

There are numerous MAS systems that have been utilised for monitoring incorporation of the *Sw-5* gene into susceptible lines in tomato breeding. These includes RFLP (restricted fragment length polymorphism), RAPD (Random amplified polymorphic DNA), RAPD-derived SCAR (Sequence characterized amplified region) and CAPS (Cleaved amplified polymorphic sequence) (Dianese *et al.*, 2010; Stevens *et al.*, 1995). To further improve the MAS system, PCR-based markers have been developed and validated for identification of the *Sw-5* gene. The system was reported to be reliable and fast and used a simple PCR assay to screen for the presence of *Sw-5* loci in resistant cultivars (Dianese *et al.*, 2010; Garland *et al.*, 2005; Shi & Vierling, 2011). Additionally, single nucleotide polymorphisms (SNPs), which are the most abundant mark in the genome and are sometimes linked to phenotypical change, have been regarded to be the most powerful molecular marker. They have become an important tool in genome mapping and tagging of important genes in plant genomics due to their abundance and high–throughput scoring potential (Caicedo *et al.*, 2007; Giancola *et al.*,

2006). In tomatoes, resistant SNP alleles have been discovered and verified and successfully used in the selection of disease resistant genotypes (Yang *et al.*, 2004).

In conclusion, this review has shown that there is an ever-growing number of tospoviruses and new vectors and host plants have been identified. Among the tospoviruses, TSWV is the most important plant virus causing high yield losses in horticultural production. Although the disease was discovered more than a century ago, it has still remained a major constraint in horticultural production worldwide. This has been attributed to its ability to infect a wide range of host plants and the presence of efficient vector species that have been shown to be difficult to manage. Understanding the genetic characteristic of isolates and the variations within vector species is important in the epidemiology of the disease and in the development of appropriate management strategies. Use of resistant cultivars has been shown to be the most efficient method for the management of the disease; however, the occurrence of resistance breaking races has further challenged its management, leading to a need for further research in alternative management option. The review has shown the potential effect of the disease in horticultural production, and now that the disease has been introduced into Africa, there is need for research to understand the disease and its vectors in order to develop appropriate mitigation measures.

Chapter 3. Distribution and genetic diversity of *Tomato spotted wilt virus* following an incursion into Kenya

3.1 Introduction

Tomato spotted wilt virus (TSWV) (genus *Tospovirus*; family *Bunyaviridae*) is one of the most widely distributed and economically important plant viruses, infecting more than 1000 host plant species in 84 families (Pappu *et al.*, 2009; Parrella *et al.*, 2003). The TSVW virion varies in size from 80 to 120 nm and has a spherical envelope. The envelopes contain one or more molecules of RNA-dependent RNA polymerase and several copies of three single-stranded negative-sense or ambisense RNA segments designated large (L), medium (M) and small (S) RNA, which are each encapsidated by the N protein. The gene for this protein is on the S RNA (Pappu, 2008; Whitfield *et al.*, 2005). The virus is transmitted by several species of thrips (*Thysanoptera*: *Thripidae*), of which western flower thrips (WFT), *Frankliniella occidentalis* (Pergande), is regarded as the most important and efficient vector (German *et al.*, 1992). *Thrips tabaci* Lindeman and *F. schultzei* (Trybom) are also common vectors of TSWV (German *et al.*, 1992). The virus is acquired only by the first larval instar, and is transmitted by the second instar just before pupation and by adults (Wijkamp *et al.*, 1993). Because the virus is not transmitted through seeds and there is no record of trans-ovarian transmission, each generation of thrips must acquire the virus for new infection to occur (van de Wetering, 1999; Wijkamp *et al.*, 1995). The virus has been reported to replicate both in the plant and in the insect body, with adults remaining viruliferous for their entire life (Nagata *et al.*, 2002; Whitfield *et al.*, 2005).

The spread of *F. occidentalis* has contributed to a worldwide resurgence of the disease and introduction in areas where it was not previously known to occur (Kirk & Terry, 2003). The presence of crops and weeds susceptible to the virus that act as reservoirs of inoculum has been shown to be important for the continuous presence of the disease (Kahn *et al.*, 2005; Wilson, 1998). Because both the virus and its vectors have broad host ranges, TSWV has continued to cause serious losses worldwide despite efforts to manage it (Dianese *et al.*, 2011; Pappu *et al.*, 2009; Persley *et al.*, 2006).

There is limited information on the occurrence of TSWV in Africa. Based on a survey of scientists' knowledge on the disease, Dafalla (2001) reported that TSWV occurred in South Africa, Togo, Uganda, Zambia and Zimbabwe. However, there is no record of how these diagnoses were made. *Tomato spotted wilt virus* is widespread in South Africa (Thompson & van Zijl, 1996). Sivparsad and Gubba (2008) obtained partial sequences of the nucleoprotein (N) gene and found that they were similar to those from Europe, indicating a possible introduction from that continent. Ssekyewa (2006) identified TSWV at low frequency using ELISA during a survey of tomato leaf-curling viruses in Uganda in 1997–1998. No extensive studies have been carried out to establish the distribution, host range and genetic diversity of TSWV in African countries other than South Africa.

TSWV was first reported in Kenya in 1999 in the Nakuru district and identified using N-gene sequencing (Wangai *et al.*, 2001), causing yield losses of up to 80% in tomatoes. There have been no comprehensive studies undertaken on the disease since its first detection. The known vectors of TSWV *F. occidentalis*, *T. tabaci* and *F. schultzei* have been reported in various vegetable production systems in Kenya (Kasina *et al.*, 2009; Nyasani *et al.*, 2010; Waiganjo *et al.*, 2008), which would provide an opportunity for its persistence and spread. Therefore, the

current study was undertaken to establish the status of TSWV in Kenya a decade after the incursion was first reported. The distribution and genetic diversity of the virus, and perceptions of farmers about the disease, were surveyed in major tomato production areas. A phylogenetic study was also performed to compare the Kenyan isolates with those from other countries to establish their relationships.

3.2 Materials and Methods

3.2.1 Tomato production survey

A survey was undertaken between November 2012 and July 2013 in four major tomato production areas in Kenya (Fig. 3.1). The areas represented agro-ecological zones with different rainfall and temperature regimes. The areas were Kirinyaga (0.5° S, 37.3° E), Nakuru (0.3° S, 36.1° E), Loitokitok (2.9° S, 37.5° E) and Bungoma (0.6° N, 34.6° E). Tomato farms were selected randomly in each of the production areas based on the availability of the crop, varieties grown and size of the farm (Table 3.1). For each farm, data were collected on size, varieties grown and use of irrigation. Farmers were questioned for their perceptions of major production constraints, their knowledge of TSWV and its vectors, and their use of control methods for thrips (Appendix 1a).

On each farm, about 50 plants were randomly examined along an X-shaped transect for virus symptoms. Leaves and fruit showing symptoms characteristic of TSWV infection were collected from each of the tomato fields. Asymptomatic samples were collected in cases where no symptomatic plants were observed. Between 1 and 14 (median 3) samples were collected from each farm depending on size. These were supplemented with samples from local markets (Table 3.1). Samples were stored at −80°C before analysis.

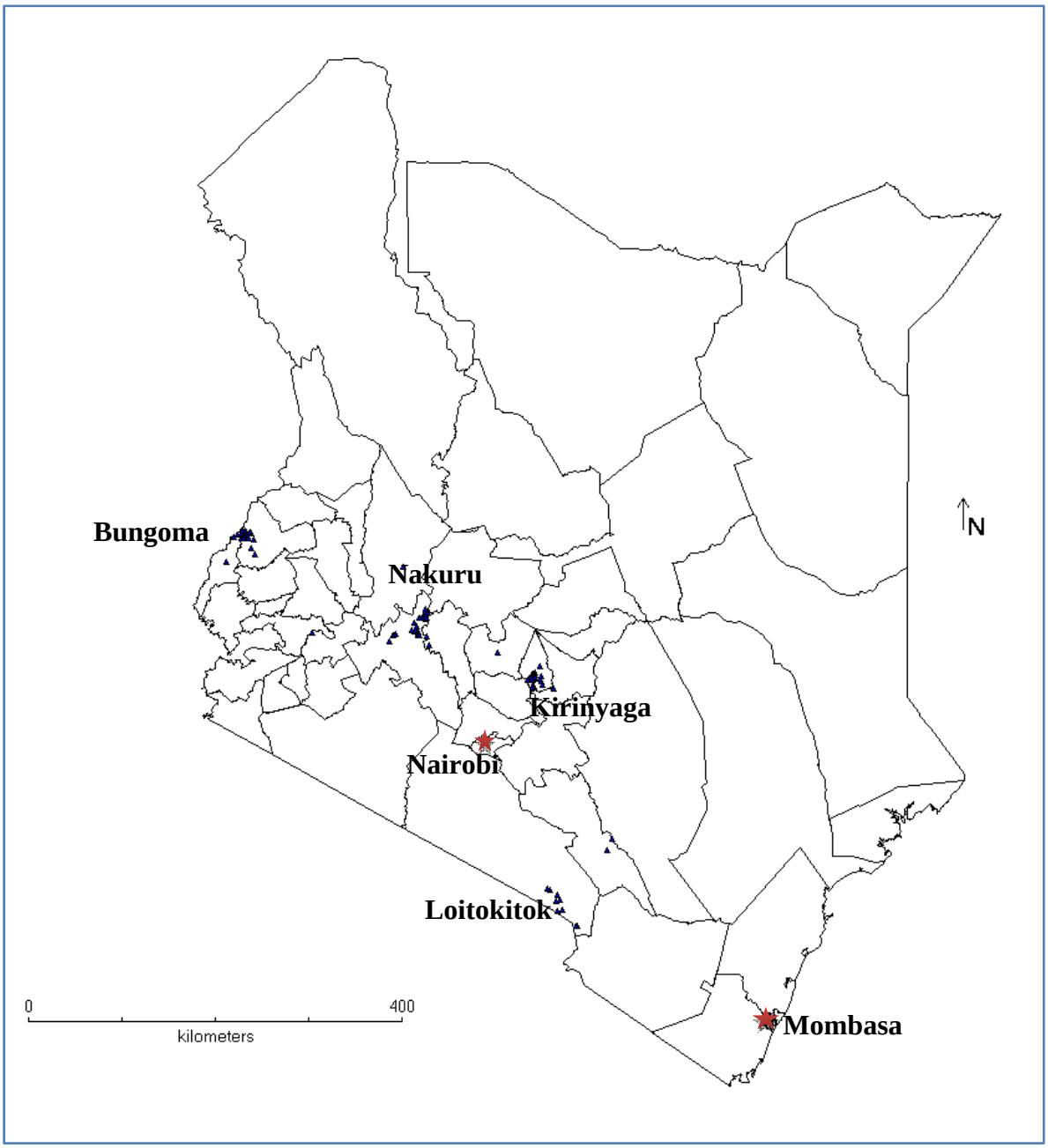

Figure 3.1 Map of Kenya indicating the four tomato production areas where sampling for *Tomato spotted wilt virus* was undertaken.

3.2.2 Serological analysis for TSWV

Most of the symptomatic samples collected during the survey were tested using quick diagnostic kits (ImmunoStrip ISK 39300, Agdia, Elkhart, IN, USA) and double antibody sandwich (DAS) ELISA (Complete Kit PSA 39300, Agdia, Elkhart, IN, USA) both of which

contained the same polyclonal antibodies specific to TSWV. Samples from Nakuru and Kirinyaga were mostly tested using quick kits, while samples from Bungoma and Loitokitok were mostly tested using ELISA. For the samples tested using ImmunoStrip, a small amount of tomato leaf or fruit was place in the extraction bag containing buffer and ground before an ImmunoStrip was placed in the bag and observed for 5–30 minutes. Occurrence of two lines on the ImmunoStrip indicated the sample was positive for TSWV

Table 3.1 Number of tomato farms and market collections surveyed in four areas of Kenya for *Tomato spotted wilt virus*, and number tested with each detection method [a]

Area	**Farms**	**Markets**	**Number tested**	**Quick kit[b]**	**DAS-ELISA[c]**	**PCR**		
						722 & 723[d]	**TSW 1& 2[e]**	**ATG & ENDE[f]**
Nakuru	48	8	50	49	16	19	31	16
Loitokitok	15	1	15	10	14	14	14	14
Bungoma	22	2	22	13	22	22	22	13
Kirinyaga	31	1	19	16	9	19	0	0
Total	116	12	106	88	61	74	67	43

DAS-ELISA, double antibody sandwich enzyme-linked immunosorbent assay; PCR, polymerase chain reaction.

[a]For each farm or market collection that was tested, up to 14 (median 3) samples were tested by at least one detection method

[b]ImmunoStrip ISK 39300, Agdia

[c]Complete Kit PSA 39300, Agdia

[d]Adkins & Rosskopf, (2002)

[e]Roberts *et al.* (2000)

[f]Grund *et al.* (2010)

For DAS-ELISA, sap was extracted by grinding plant samples in general extraction buffer (sodium sulfite 1.3 g L^{-1}, polyvinylpyrrolidone (PVP) 20.0 g L^{-1}, sodium azide 0.2 g L^{-1},

powdered egg albumin 2.0 g L^{-1}, Tween 20 20.0 g L^{-1}, pH 7.4) provided in the kit at a ratio of 1:10 (tissue to buffer), and 100 µl of the sample was loaded into a well in a microtitre plate coated with TSWV specific antibody. Positive and negative controls were also included. The plate was processed according to the manufacturer's protocol. Briefly, the plate was incubated overnight at 4°C, after which it was washed with phosphate buffered saline-Tween 20 (PBS-T) buffer. An aliquot of 100 µl of alkaline phosphatase labelled antibody conjugate diluted 1:100 was added to each well in the microtitre plate and incubated for 2 hours at room temperature. The plate was then washed with PSB-T before addition of 100 µl p-nitrophenyl phosphate in substrate buffer to each well. The plate was incubated for colour development for one hour at room temperature. Absorbance (A_{405}) was recorded after 1 hour incubation using a microplate reader (FLX 800, BioTEK, VT, USA). Samples with absorbance more than three times the average negative control were considered positive.

Symptomatic samples that tested negative for TSWV were subjected to further analysis with DAS-ELISA using virus specific antibodies for other tospoviruses including *Groundnut bud necrotic virus* (GBNV), *Groundnut ringspot virus* (GRSV), *Impatiens necrotic spot virus* (INSV), *Tomato chlorotic spot virus* TCSV) and *Watermelon silver mosaic virus* (WSMoV) from Agdia (USA), and *Capsicum chlorosis virus* (CaCV), *Chrysanthemum stem necrosis virus* (CSNV), and *Tomato yellow ring virus* (TYRV) from DSMZ (Braunschweig, Germany) according to the manufacturers' protocols. The symptomatic samples that tested negative were also assayed for *Cucumber mosaic virus* (CMV) and *Pepino mosaic virus* (PepMV) using DAS-ELISA, because these viruses produce symptoms similar to what was observed. A total of 196 samples were assayed from Nakuru (43), Kirinyaga (23), Loitokitok (40) and Bungoma (90) for all 10 of these viruses.

3.2.3 Inoculation on indicator plants

Four randomly selected positive samples were mechanically inoculated on a range of indicator plants to determine disease expression. The samples were inoculated on *Petunia hybrida, Nicotiana glutinosa*, *Nicotiana benthamiana, Nicotiana tabacum*, *Capsicum annuum*, *Chenopodium amaranticolor, Chenopodium quinoa* and *Datura stramonium*. Tomato and cowpeas (*Vigna unguiculata*) were also inoculated. The indicator plants were maintained in an insect-proof greenhouse and observed for symptom expression. The plants were then assayed using DAS-ELISA to confirm infection with TSWV.

3.2.4 Molecular identification using reverse transcriptase-polymerase chain reaction

Tomato samples, both symptomatic and asymptomatic, were assayed for TSWV using reverse transcriptase-polymerase chain reaction (RT-PCR). A total of 333 samples from Nakuru (118), Kirinyaga (70), Loitokitok (47) and Bungoma (98) were assayed.

RNA was extracted from fruits and leaves using ZR Plant RNA MiniPrep kits (Zymo Research Corporation, CA, USA) according to the manufacturer's protocol. Plant materials were ground in liquid nitrogen and 100 mg was transferred to 2 ml tubes for processing. The quality and quantity of the extracted RNA was determined using a NanoDrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The resulting RNA was stored at −80°C.

cDNA synthesis was undertaken using a Maxima First Strand cDNA synthesis kit for RT-qPCR (Thermo Scientific) containing oligo (dT) and random hexamer primers. cDNA synthesis was conducted according to the manufacturer's protocol with some modification. A

20 µl reaction consisting of 4 µl of reaction buffer mix, 2 µl of Maxima enzyme mix, 2 µl RNA and nuclease–free water was performed. The mixture was incubated for 10 min at 25°C, followed by 30 min at 50°C and the reaction was terminated by heating the mixture at 85°C for 15 min. cDNA was stored at −20°C until required.

Polymerase chain reaction was undertaken using several primer sets (Table 3.2), including both specific and degenerate primers to establish occurrence of TSWV and other tospoviruses. Primer sets TSWV 722 and TSWV 723 (Adkins & Rosskopf, 2002), TSW 1 and TSW 2 (Roberts *et al.*, 2000), and TSWV-N-ATG and TSWV-N-Ende (Grund *et al.*, 2010) were specific for TSWV. The three primer sets were used for comparison in case a virus isolate was not amplified with one of the sets. The degenerate primer pair gM410 and gM870c (T. C. Chen *et al.*, 2012) and primers for INSV (Mumford *et al.*, 1996) and TYRV (Birithia *et al.*, 2012) were used to detect tospoviruses in symptomatic plants that were negative for TSWV, particularly from Bungoma and Loitokitok. GAPDH primers designed from *Solanum lycopersicum* glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from GenBank sequence NM-001279325 using primer3, were used as internal controls to check the quality of RNA used in the analysis. Polymarese chain reactions were performed using AccuPower PCR Premix (Bioneer Corporation, Daejeon, Korea). Two microlitres of cDNA in a 20 µl reaction was used with a final concentration of 1 × buffer, 1.5 mM $MgCl_2$, 250 µM dNTPs, 0.8 mM forward and reverse primer and 1 U Taq polymerase. Amplification was carried out in an Applied Biosystems 9700 thermocycler (Applied Biosystems, Foster City, CA, US) with the following profile: initial denaturation at 95 °C for 3 minutes; 35 cycles of denaturation at 94 °C for 45 seconds, annealing at the appropriate temperature for each primer pair (Table 3.2) for 45 seconds and extension of 72 °C for 1 minute; and final extension at 72°C for 10

minutes. The PCR products were visualised by electrophoresis in a 2% agarose gel stained with GelRed (Biotium, Hayward, CA, USA).

3.2.5 Phylogenetic analysis

Polymerase chain reaction products obtained using the TSWV 722 and TSWV 723 primers were purified using a QIAquick DNA purification kit (Qiagen, Hilden, Germany). DNA sequences were generated using the same primers on an ABI 3370 sequencer (Applied Biosystems, Foster City, CA, USA) at Bioscience Eastern and Central Africa (BecA-ILRI) Hub sequencing unit. Forward and reverse sequences were obtained for each sample.

Partial N-gene nucleotide sequences were trimmed and assembled using CLC Workbench software (CLC Bio, Aarhus, Denmark). For each sample, the overlapping portions of high-quality read from the forward and reverse sequences were used to build a consensus sequence 473 nucleotides long. The resulting consensus sequences were aligned using ClustalW and used for phylogenetic analysis in MEGA 5 (Tamura *et al.*, 2011). Genetic distances were calculated using the Kimura 2 parameter distance model. Neighbour-joining and maximum likelihood trees were constructed with 1000 bootstraps. Because of the large numbers of identical sequences that were obtained, only selected sequences were used to construct the phylogenetic trees. Representative databased sequences similar to the sequences obtained in this study were included in the phylogenetic trees.

Table 3.2 A list of primers used for reverse transcription-polymerase chain reaction (PCR) in the detection of *Tomato spotted wilt virus* and other tospoviruses

Target	**Name of primer**	**Amplicon size (bp)**	**Primer sequence**	**Annealing temperature (°C)**	**Reference/source**
TSWV	TSWV-722	620	GCTGGAGCTAAGTATAGCAGC	55	Adkins & Rosskopf, (2002)
	TSWV-723		CACAAGGCAAAGACCTTGAG		
TSWV	TSW 1	628	TCTGGTAGCATTCAACTTCAA	45	Roberts *et al.* (2000)
	TSW 2		GTTTCACTGTAATGTTCCATAG		
TSWV	TSWV-N-ATG	650	ATGTCTAAGCTTAAGCTCAC	60	Grund *et al.* (2010)
	TSWV-N-Ende		GCTGGAGCTGAGTATAGC		
Tospovirus	gM410	500	AACTGGAAAAATGATTYNYTTGTTGG	52	Chen *et al.* (2012)
	gM870c		ATTAGYTTGCAKGCTTCAATNAARGC		
INSV	S1 INSV F	602	AAATCAATAGTAGCATTA	55	Mumford *et al.* (1996)
	S2 INSV R		CTTCCTCAAGAATAGGCA		
TYRV	TFfor	912	ACTCATTAAAATGCATCGTTCT	52	Birithia *et al.* (2012)
	TFrev		CTAAGTAAACACCATGGCTACC		
Internal gene	GAPDH F	544	ATGACTGCCACCCAGAAAAC	55	Designed from GAPDH gene using Primer3.
	GAPDH R		CTCCAGGCCCACAAAACTAA		

3.3 Results

The survey revealed that tomato production occurred mainly during the dry season in each area to minimise the risk of foliar diseases such as late blight. Of the crops surveyed, 71% were at the harvesting stage, 13% were fruiting but not yet being harvested, and 16% were flowering or in vegetative stages. Crops at Kirinyaga tended to be less mature than in the other areas, with only 45% at harvest stage. The majority (59%) of the farmers relied on irrigation, except in the Bungoma area where they relied on rainfall. Over 67% of the farms were less than 1 ha in area. The major constraints to tomato production were reported as pests and diseases. Only 34% of the farmers were aware of the occurrence of thrips in tomato production, but 95% of these farmers used chemical control for their management. Farmers without knowledge of thrips did not apply any control measures. Low knowledge of thrips occurrence was recorded in Kirinyaga and Nakuru areas with the highest awareness (93%) occurring in the Loitokitok area. The farmers in these areas also had the highest awareness of TSWV (87%) compared with 40% awareness overall. TSWV was referred to in Swahili as ‘jeshi’, ‘sura mbili’ and ‘kitenge’. Disease incidence was reported to have been low in the 2012/2013 cropping season compared to previous seasons.

In the Nakuru area where the disease was found to be endemic, similar observations were made with a high percentage (60%) of farmers not being aware of the thrips and its role in the transmission of the virus. Farmers who were not aware of the thrips represented a high proportion (82%) of farms that had samples that tested positive. Consequently, 91% of the farms with samples that tested positive were also found not to have applied chemicals for the control of thrips.

At least 13 tomato cultivars were grown in different production areas. However, Rio Grande, Kilele and Oxly were the most cultivated varieties and only 4% of farms did not have one of these as their major variety. Rio Grande was the main variety on more than 59% of farms and was the only variety that was being cultivated in all four production areas. Rio Grande was particularly the most preferred variety in Nakuru and Loitokitok. Kilele was the second most widely grown tomato cultivar and most preferred in Kirinyaga, while Oxly was the main variety in Bungoma. None of the varieties being cultivated were reported to be resistant or tolerant to TSWV.

Symptoms similar to those caused by TSWV were observed on tomato in all production areas. Concentric rings and uneven ripening, necrosis and purpling were the most commonly encountered symptoms. Other viral symptoms observed included yellowing, leaf curl and mosaic. Out of 408 samples collected, 89 (22 %) tested positive for TSWV by at least one detection method. Most of these were from the Nakuru area (Table 3.3). Over 43% of the samples that were collected from Nakuru, from 55% of farms, tested positive for TSWV. Only one sample from Loitokitok tested positive. *Tomato spotted wilt virus* was observed in all divisions in Nakuru that had major tomato production activities with the highest number of positive samples reported in Subukia division where the disease was first reported.

A high incidence of tomato plants with symptoms resembling those of TSWV, mainly on fruits, were observed in the Bungoma area. However, the samples did not react with TSWV antibodies or with antibodies from other tospoviruses including INSV, WSMoV, GRSV, TCSV, CSNV and TYRV. The samples also tested negative in RT-PCR against TSWV using the three sets of specific primers, and to other tospoviruses using degenerate primers. A smaller set of samples that were tested for the tospoviruses INSV and TYRV were also

negative. Similarly, samples collected from Kirinyaga tested negative for TSWV and other tospoviruses.

Table 3.3 Result of testing for *Tomato spotted wilt virus* in tomato samples collected from four major tomato production areas in Kenya[a].

Area	Division	No. of samples	No. positive
Nakuru	Bahati	70	26
	Njoro	6	0
	Solai	29	15
	Subukia	94	47
Kirinyaga	Kerugoya	7	0
	Mwea	76	0
Loitokitok	-	44	1
Bungoma	Cheptais	14	0
	Malakisi	23	0
	Sirisia	45	0
Total		408	89

[a]A sample was recorded as positive if it gave a positive reaction to at least one immunoassay [double antibody sandwich enzyme-linked immunosorbant assay (DAS-ELISA) or Immunostrip] or reverse transcriptase-polymerase chain reaction (RT-PCR) test

Collected samples were assayed using different methods that included immunostrips, ELISA and RT-PCR. Analysis of 103 samples from Nakuru that were tested using both serology (Immunostrip or DAS-ELISA) and RT-PCR tests showed that all 52 samples that were positive with serology also tested positive with RT-PCR. However, one third (17) of samples that tested negative with serology were positive with RT-PCR, indicating the greater sensitivity of the molecular test.

Selected samples that were positive to TSWV produced varied symptoms in a range of indicator plants within 4–14 days after inoculation. *C. amaranticolor* and *Petunia hybridus* produced local symptoms (necrotic spots), while *V. unguiculata, C. annuum, D. stramonium, S. lycopersicum* and *N. benthamiana* mainly produced systemic symptoms including mosaic, mottling, leaf distortion, leaf death, wilting, stunting, purpling, ring spot, chlorosis and necrosis (Table 3.4). Symptomatic indicator plants tested positive with DAS-ELISA, indicating that the observed symptoms were due to TSWV infection.

Table 3.4 Symptoms of selected *Tomato spotted wilt virus* isolates collected from Nakuru and Loitokitok on indicator plants

Host	**Common name**	**Type of symptom**	
		Local	**Systemic**
Chenopodiaceae			
Chenopodium quinoa	Quinoa	-	-
Chenopodium amaranticolor	Chenopodium	NS	-
Fabaceae			
Vigna unguiculata	Cowpea	NR	MO, CS
Solanaceae			
Capsicum annuum	Pepper	CS	MO, LD
Datura stramonium	Thorn apple	NS	MO, CN, LD, M, C, ST, SN, W
Solanum lycopersicum	Tomato (Moneymaker)	-	M, LD, ST, P, C
Nicotianum benthamiana	Tobacco	CN	MO, W, M, NS, ST
Nicotiana glutinosa	Tobacco	-	-
Nicotiana tabacum	Tobacco	-	-
Petunia hydrida	Petunia	NR	

C, chlorosis; CN, chlorotic ringspot; CS, chlorotic spot; LD, leaf deformation; M, mosaic; MO, mottle; NR, necrotic ringspot; NS, necrotic spot; P, purpling; SN, severe necrosis; ST, stunting; W, wilting; -, no symptoms.

Partial N-protein gene sequences were obtained from 45 selected isolates of TSWV from tomato leaves and fruits. The nucleotide sequences have been deposited in GenBank with accession numbers KF963116-127 and KF964053-062 (Appendix, Table A1). The BLAST results confirmed that the isolates were TSWV, based on the high level of sequence similarity (97–99%) with TSWV sequences in GenBank from isolates from other countries. The sequences from the current study showed high sequence identity (99–100%) among themselves. The phylogenetic relatedness among 10 isolates, which represented all unique sequences that were found, and 75 nucleotide sequences from GenBank was examined. The

analysis produced three independent clusters consisting of group I with sequences from Europe, Australia, South Africa and North Carolina; group II (Japan and Korea) and group III (USA, Brazil and Spain) (Fig. 3.2). The Kenyan isolates clustered with group I but formed a single subgroup distinct from other isolates in this group. Although all sequences available in GenBank from South Africa also clustered in the group I, they were not closely related to the Kenyan isolates.

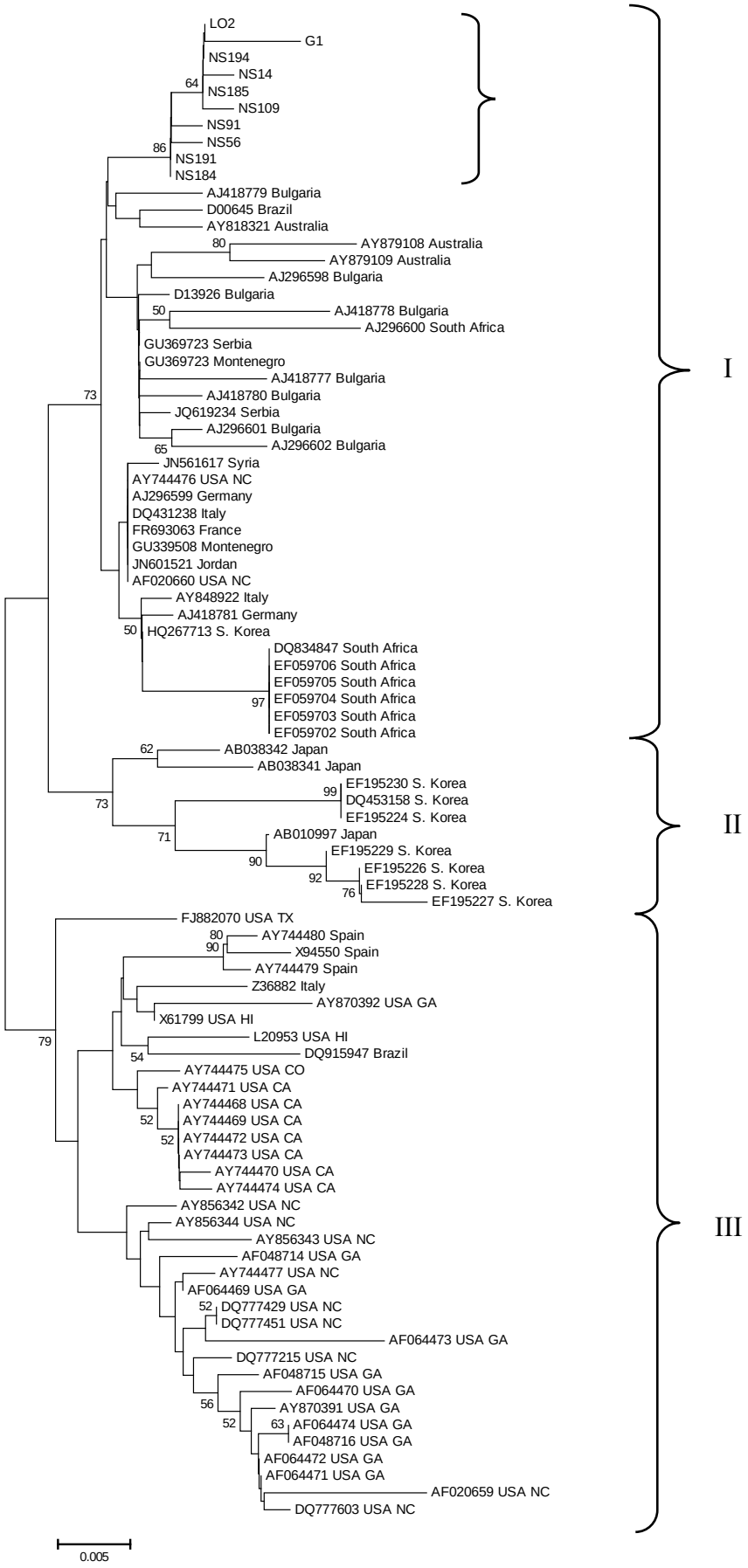
LO2
G1
NS194
NS14
NS185
NS109
NS91
NS56
NS191
NS184
AJ418779 Bulgaria
D00645 Brazil
AY818321 Australia
AY879108 Australia
AY879109 Australia
AJ296598 Bulgaria
D13926 Bulgaria
AJ418778 Bulgaria
AJ296600 South Africa
GU369723 Serbia
GU369723 Montenegro
AJ418777 Bulgaria
AJ418780 Bulgaria
JQ619234 Serbia
AJ296601 Bulgaria
AJ296602 Bulgaria
JN561617 Syria
AY744476 USA NC
AJ296599 Germany
DQ431238 Italy
FR693063 France
GU339508 Montenegro
JN601521 Jordan
AF020660 USA NC
AY848922 Italy
AJ418781 Germany
HQ267713 S. Korea
DQ834847 South Africa
EF059706 South Africa
EF059705 South Africa
EF059704 South Africa
EF059703 South Africa
EF059702 South Africa
AB038342 Japan
AB038341 Japan
EF195230 S. Korea
DQ453158 S. Korea
EF195224 S. Korea
AB010997 Japan
EF195229 S. Korea
EF195226 S. Korea
EF195228 S. Korea
EF195227 S. Korea
FJ882070 USA TX
AY744480 Spain
X94550 Spain
AY744479 Spain
Z36882 Italy
AY870392 USA GA
X61799 USA HI
L20953 USA HI
DQ915947 Brazil
AY744475 USA CO
AY744471 USA CA
AY744468 USA CA
AY744469 USA CA
AY744472 USA CA
AY744473 USA CA
AY744470 USA CA
AY744474 USA CA
AY856342 USA NC
AY856344 USA NC
AY856343 USA NC
AF048714 USA GA
AY744477 USA NC
AF064469 USA GA
DQ777429 USA NC
DQ777451 USA NC
AF064473 USA GA
DQ777215 USA NC
AF048715 USA GA
AF064470 USA GA
AY870391 USA GA
AF064474 USA GA
AF048716 USA GA
AF064472 USA GA
AF064471 USA GA
AF020659 USA NC
DQ777603 USA NC
0.005
I
II
III

Figure 3.2 Phylogenetic relationships of partial N-gene sequences from *Tomato spotted wilt virus* isolates collected from tomato production areas in Kenya and databased sequences from other geographic regions. All sequences were trimmed to 473 nucleotides. The tree was drawn using the Kimura 2-parameter distance measure and the neighbour-joining method with 1000 bootstrap replicates in MEGA 5. Kenyan isolates are labelled G (samples from Nakuru raised on *D. stramonium* in the greenhouse), NS (samples from Nakuru) and LO (sample from Loitokitok). Only selected sequences from Kenya are shown in the tree

3.4 Discussion

This study has revealed the continued presence of TSWV in the Nakuru area more than a decade after it was first reported (Wangai *et al.*, 2001). The disease was found to occur in all the major tomato production divisions within this area. Despite its widespread occurrence, there was low awareness of the virus, its vectors and alternative hosts. This is possibly because occurrence of severe disease is more sporadic than for the other major diseases such as late blight and bacterial wilt. Because of this low awareness, there was limited effort by farmers to manage the vectors. Chemical control was the sole option that was being utilised in the management of thrip vectors. Chemical control has been reported as one of the important and widely used method for the management of thrips, but its effectiveness is unknown. Thrips control can be difficult to assess because of its cryptic feeding behaviour and resistance to chemicals (Gao *et al.*, 2012; Momol *et al.*, 2004). Occurrence of efficient vectors within tomato production at Nakuru was observed in the study on diversity of thrips species (Macharia, *et al.*, 2015b). Cropping at Nakuru is based on a diverse range of vegetables and other crops in both wet and dry seasons and these, together with weeds, include a number of alternative hosts for both the virus and its vectors. This could have contributed to the persistence of the disease in the Nakuru area.

There had been limited dispersal of TSWV away from Nakuru. This may reflect the geographic separation of the areas, and limited trade between them of potentially infected plant material. The only other production area where it was positively identified was Loitokitok. This is approximately 300 km southeast of Nakuru and is separated from other farming areas by semiarid rangeland. Only one sample tested positive, indicating a low prevalence of disease. This reflected the high level of awareness of the disease by growers and the high level of chemical control for thrips.

Samples from the Bungoma area tested negative to TSWV despite having symptoms resembling those of tomato spotted wilt. No known vectors were identified among thrips samples collected from the Bungoma area during this or previous surveys (Macharia, *et al.*, 2015b). However, Ssekyewa (2006) reported the occurrence of ELISA-positive TSWV in the Mbale district in Uganda which is located close to Bungoma. These symptoms indicate the possible occurrence of other tomato viruses or a different strain of TSWV on tomatoes in Bungoma. Birithia *et al.* (2012) reported the occurrence of the recently recognised tospovirus TYRV at Loitokitok, causing symptoms similar to those of TSWV. However, we were unable to detect TYRV by either ELISA or RT-PCR. Testing of the symptomatic samples from Bungoma for a range of tospoviruses and other common tomato viruses failed to give a positive result. Therefore, there is a need to carry out further work to establish the cause of the symptoms observed.

The genetic diversity of Kenyan samples established through phylogenetic analysis based on the N-protein indicated that they were similar to TSWV isolates reported from other countries. The analysis indicated formation of three major groups. These consisted mostly of

European, Asian and American isolates respectively, although group I also contained isolates from Australia and Africa. The basic pattern was similar to those found in previous studies based on the N-gene (Hagen *et al.*, 2011; Sivparsad & Gubba, 2008; Tentchev *et al.*, 2011; Timmerman-Vaughan *et al.*, 2014; Tsompana *et al.*, 2005), but differed in detail because of the different number of sequences used. The analysis confirmed that isolates were clustered based on geographical occurrence rather than host plants. Each of the three groups contained isolates from tomato, other Solanaceae, Asteraceae, and a range of other plant species.

It is believed that the worldwide spread of WFT has contributed to severe outbreaks of TSWV in areas where it was not previously regarded as an important disease (Kirk & Terry, 2003). Tentchev *et al.* (2011) suggested two mechanisms for the emergence of TSWV. One was that TSWV is introduced into an area with the WFT vector, and the other is that the introduction of WFT allows the re-emergence of an indigenous population of TSWV. Western flower thrips was first detected in Kenya in 1989 (Kedera & Kuria, 2003), 10 years before the virus was first detected (Wangai *et al.*, 2001). This suggests a third pattern, where TSWV was introduced into an area that had an established population of vectors. The Kenyan isolates clustered with group I where they formed a single sub-clade. The limited sequence diversity of TSWV in Kenya suggests a single recent introduction with some local diversification since then. The actual origin of the Kenyan incursion is uncertain. The Kenyan isolates were not closely related to those from the long-established South African population, suggesting an introduction from outside Africa, possibly from Europe. The lack of evidence for spread from South Africa to Kenya has implications for quarantine within the African continent.

The analysis of samples using serological (Immunostrips and ELISA) and RT-PCR methods indicated their relative reliability in identification of TSWV. Immunostrips which are easy to

use and require no equipment were found to be comparable to DAS-ELISA but less sensitive than RT-PCR. In previous studies Immunostrip and RT-PCR gave similar results when potatoes were tested for TSWV (Abad *et al.*, 2005; Tsompana *et al.*, 2005), while ELISA was found to be comparable with RT-PCR in detecting TSWV in peanut (Dang *et al.*, 2009). On the other hand, real-time RT-PCR was found to be more sensitive than ELISA for detecting TSWV in bulked samples of several plants and parts of leaves with low virus titre (Dietzgen *et al.*, 2005). In our experience, Immunostrip was useful for undertaking quick analysis during field monitoring and surveillance. DAS-ELISA using polyclonal antibodies had the lowest cost per sample of the methods that we used, but had a relatively high risk of false negative results. This could be countered by testing more samples from a plant or field to allow for spatial variation in virus titre. RT-PCR was the most sensitive and reliable method but had the highest cost.

In conclusion, the study has revealed limited distribution of TSWV in Kenya. TSWV has persisted in the area where it was first reported. Low awareness of the virus, its vectors and alternative hosts could have contributed to this persistence. This study suggests the need for further research to establish the cause of symptoms observed on tomatoes in Bungoma and determine the impact of TSWV on other vegetable crops, field crops and ornamental plants being cultivated in Kenya. The current study employed a variety of approaches to survey the occurrence of TSWV in the Kenyan tomato production regions. This will lay a foundation for further research on the identification and distribution of the virus in Kenya, and for effective control of the disease and management of tomato production in the farming system of the country.

Chapter 4. Diversity of thrips species and vectors of *Tomato spotted wilt virus* in tomato production systems in Kenya

4.1 Introduction

Thrips (Thysanoptera: Thripidae) are among the most economically important pests in crop production systems. They can cause physical damage on leaves and fruits and act as important vectors of viruses in vegetable and ornamental production. Most thrips species are polyphagous, attacking both perennial and annual crops, hence supporting their survival and invasiveness. They have been reported as the sole vectors of tospoviruses with at least 14 thrips species consisting of eight *Frankliniella* species, three *Thrips* species, and one species of each *Scirtothrips*, *Dictyothrips,* and *Ceratothripoides* shown as important vectors of tospoviruses (Riley *et al.*, 2011b). Among these, nine thrips species are vectors of *Tomato spotted wilt virus* (TSWV): *Frankliniella occidentalis* (Pergande) (Western flower thrips), *Frankliniella schultzei* (Trybom) (common blossom thrips), *Frankliniella fusca* (Hinds) (tobacco thrips), *Frankliniella bispinosa,* (Morgan), *Frankliniella intonsa* (Trybom), *Frankliniella cephalica* (Crawford), *Frankliniella gemina* Bagnall, *Thrips tabaci* Lindeman (onion thrips), and *Thrips setosus* Moulton (Pappu *et al.*, 2009; Riley *et al.*, 2011b).

F. occidentalis has been reported to be the most efficient vector and readily retains acquired virus throughout its life (Wijkamp *et al.*, 1995). Its high fecundity, efficient and rapid dispersal ability and polyphagous nature enable even a small number of western flower thrips to transmit TSWV to a substantial number of plants (Reitz, 2009; Riley *et al.*, 2011b). *F.*

occidentalis has also been reported as a vector of other tospoviruses including *Impatiens necrotic spot virus* (INSV), *Chrysanthemum stem necrosis virus* (CSNV), *Groundnut ringspot virus* (GRSV) and *Tomato chlorotic spot virus* (TCSV) (Pappu *et al.*, 2009; Riley *et al.*, 2011b).

Transmission studies have shown variation in the competence of *T. tabaci* as a vector of TSWV (Jacobson *et al.*, 2013; Westmore *et al.*, 2013). Both poor and efficient transmitting populations have been observed in Europe, Australia and the United States (Chatzivassiliou *et al.*, 2000; Jacobson & Kennedy, 2013; Jenser *et al.*, 2003; Westmore *et al.*, 2013; Wilson, 2001). Similar observations were reported with *F. schultzei* where the dark form was considered to be a more prolific vector of tospoviruses than the pale form (Sakimura, 1969; Sakurai, 2004; Wijkamp *et al.*, 1995). Furthermore, variation observed within *F. occidentalis* was reported to affect their reaction to insecticides (Brødsgaard, 1994; Jensen, 2000). Therefore, discrimination of thrips species is important in understanding their contribution to transmission of tospoviruses and in development of appropriate management systems.

Molecular identification of thrips has been used in evaluation of variation that occurs within populations (Brunner *et al.*, 2004; Kadirvel *et al.*, 2013). Based on mitochondrial cytochrome oxidase 1 (CO1) sequences, *T. tabaci* was classified into three groups (Brunner *et al.*, 2004). These comprised a group from tobacco, and two polyphagous forms that differed in reproduction: arrhenotokous, where parthenogenetically produced progeny are males; and thelytokous, where parthenogenetically produced progeny are females (Brunner *et al.*, 2004; Jacobson & Kennedy, 2013; S. Toda & Murai, 2007). Similar observations were made with *F. occidentalis* where the species was divided into two genetically divergent groups labelled L and G which may represent cryptic species (Rugman-Jones *et al.*, 2010). Analysis of the CO1

gene has also suggested that insects identified morphologically as *F. schultzei* may belong to more than one species (Kadirvel *et al.*, 2013).

TSWV was first reported in tomato in Kenya more than a decade ago (Wangai *et al.*, 2001). Since then, the disease has become a recurrent problem, and studies on its epidemiology, including thrips vectors, are essential to develop effective control strategies. Studies on thrips populations on beans in Kenya have been undertaken based on morphological identification (Kasina *et al.*, 2009; Nyasani *et al.*, 2010). However, morphological techniques cannot identify cryptic species or subspecies that may differ in their biology and vector competence. Kadirvel *et al.* (2013) has demonstrated the feasibility of CO1 barcoding for identifying thrips populations in vegetable crops, whereas Timm *et al.* (2008) have published a molecular key, based on CO1 sequences, for major pest species of thrips in southern Africa. However, no studies have been undertaken so far to determine the thrips diversity and occurrence of vectors of TSWV in tomato production using molecular techniques. The objectives of the current study were to 1) determine the diversity of thrips species and 2) establish the occurrence of vectors of TSWV in four selected major tomato production areas in Kenya.

4.2 Materials and Methods

Thrips samples were collected between December 2012 and June 2013 from major tomato production areas in Kenya. The selected period represent the major tomato growing season in the selected areas. Selected areas represented different agro-ecological zones with different rainfall and temperature regimes. The areas were Kirinyaga (Central Province, coordinates 0.5° S, 37.3° E), Nakuru and Loitokitok (Rift Valley Province, coordinates 0.3° S, 36.1° E; and 2.9° S, 37.5° E) and Bungoma (Western Province, near the Ugandan border, coordinates

0.6° N, 34.6° E). Thrips samples were collected from tomato and major weeds occurring within tomato production with the aim of identifying alternative hosts. At least 25 plants were randomly examined for occurrence of thrips species along an X-shaped transect in each tomato field. Thrips were collected by tapping the plants on a white surface and picking the thrips using a soft camel hair brush into a vial containing 80% ethanol. The samples were later separated under a stereomicroscope into morphologically similar groups according to the plant species they were collected from in each farm and stored at 4°C before molecular identification.

DNA was extracted from individual thrips using the Qiagen DNeasy Blood and Tissue kits (QIAGEN, Hilden, Germany). Thrips were transferred into 1.5 ml microcentrifuge tubes and ethanol was removed by pipetting. Thrips were homogenised using a micropestle and DNA extraction was performed according to the manufacturer's specification for DNA extraction from tissue. Extracted DNA was stored at -20°C.

Cytochrome oxidase 1 primer pairs, forward primer LCO 1490 (5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′) and reverse primer HCO 2198 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′) were used to amplify the partial mitochondrial CO1 gene (Hebert *et al.*, 2003). Polymerase chain reaction (PCR) reactions were performed using AccuPower Taq PCR PreMix (Bioneer Corporation, Daejeon, Korea) with increased $MgCl_2$ concentration. In total, 3–5 µl of DNA extract in a 20 µl reaction was used with a final concentration of 1 × PCR buffer, 1.9 mM $MgCl_2$, 250 µM dNTPs, 10 µM LCO and HCO primers, and 1 U Taq polymerase. Amplification was carried out in an Applied Biosystems 9700 thermocycler (Foster City, CA) with the following profile: initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 30 s and extension at

72°C for 90 s. The final extension was undertaken at 72°C for 10 min. The expected amplicon size was 658 bp. The PCR products were visualised in a 2% agarose gel stained with GelRed (Biotium, Hayward, CA).

PCR products were purified using QIAquick PCR purification kits (QIAGEN, Hilden, Germany). DNA sequences were generated using the PCR primers on an ABI 3370 Genetic Analyzer (Applied Biosystems, Foster City, CA) at Biosciences eastern and central Africa-International Livestock Research Institute Hub's (BecA-ILRI Hub) sequencing unit. The sequencing was performed for both strands of the fragments for all the samples.

The presence of TSWV in adult thrips species was established through analysis of 58 single thrips samples randomly selected from the Nakuru area. Because this is an RNA virus, the DNA extractions used for barcoding were unsuitable and nucleic acid was extracted from single thrips using a simple Chelex 100 resin-based method according to Boonham *et al.* (2002) with some modifications. A single thrips was transferred into a 1.5 mL microcentrifuge tube and residual ethanol removed by pipetting. Nuclease-free water (50 µL) was added and the thrips ground using a micropestle. Fifty microliters of Chelex solution (slurry of Chelex 100 resin: nuclease-free water at a ratio of 1:5) was added to the mixture and incubated at 95°C for 5 min. The tubes were centrifuged at 11,000 rpm for 5 min. The supernatant was transferred to a new tube and stored at -20°C until required for RT-PCR amplification. Nucleic acid was also extracted from symptomatic tomato previously identified to be infected with TSWV for comparison.

Sequencing of a fragment of the N protein with TSWV-N-ATG (5'-ATG TCT AAG CTT AAG CTC AC-3') and TSWV-N-Ende (5'-GCT GGA GCT GAG TAT AGC-3') primers

(Grund *et al.*, 2010) was conducted and sequences were used in the identification of TSWV in thrips. One-step RT-PCR was carried out using the Qiagen OneStep RT-PCR Kit with 2 µl of nucleic acid extract in a 25 µl reaction mix of 5 µl of 5× one-step PCR buffer (containing 2.5 mM $MgCl_2$), 400 µM dNTP mix, 0.4 µM of forward and reverse primers and 1 µl of one-step RT-PCR enzyme mix (containing reverse transcriptase and Hotstar Taq DNA polymerase). The reactions were performed as follows: reverse transcription at 48°C for 45 min; 95°C for 15 min to activate the Hotstar Taq DNA polymerase and inactivate reverse transcriptase; 35 cycles of 94°C for 30 s denaturation of cDNA, 60°C for 1 min annealing and 72°C for 1 min extension; and a final extension at 72°C for 10 min. The resulting PCR products were visualised in a 2% agarose gel stained with GelRed and the expected size was 650 bp.

The sequences from both thrips and TSWV were assembled using CLC workbench software (CLC Bio, Aarhus, Denmark). For each sample, the overlapping portions of high-quality read from the forward and reverse sequences were used to build a consensus sequence. This was shorter than the whole amplicon but gave a more accurate sequence. The resulting consensus sequences were compared with sequences in GenBank using the Basic Local Alignment Sequence Tool (BLASTn) and Barcode of Life Database (BOLD). The consensus sequences were aligned using ClustalW and used for phylogenetic analysis in MEGA5 (Tamura *et al.*, 2011). Genetic distances were calculated using the Kimura two-parameter distance model. Sequences were not trimmed, so that in determining the distance between any pair of sequences the maximum amount of information was used. Neighbour-joining, Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and maximum likelihood (ML) trees were constructed with bootstrapping of 1000 times. Percent sequence identity was generated using Clustal Omega multiple sequence alignment program (www.ebi.ac.uk/tools/mas/clustalo). Sequences with more than 500 high-quality base calls

were used in the phylogenetic analyses. Because of the large numbers of identical sequences that were obtained, only selected sequences of each type were used in drawing the phylogenetic trees. Representative databased sequences from species suggested by BLASTn searches to be similar to the sequences obtained in this study were included in the phylogenetic trees.

4.3 Results

In total, 164 samples of thrips were collected from the four major tomato production areas namely Nakuru (59), Kirinyaga (32), Loitokitok (35) and Bungoma (38). Some samples contained more than one morphological type, so more than one sequence was obtained from these samples. In total, 299 useable CO1 partial sequences were obtained (Table 4.1). Representative sequences have been deposited in GenBank as accessions KF640664–KF640861, KF765376–KF765384 and KF778754–KF778782 (Appendix, Table A2). BLASTn searches were used to find the sequences from named species that were most similar to the Kenyan sequences. Eight species among the Kenyan specimens were similar enough (> 95% identity) to GenBank sequences to be considered conspecific, so were identified to species (Table 4.1). UPGMA clustering of sequences was used to suggest the relationships of those specimens that were more dissimilar to databased sequences (Appendix, Fig. A1). A number of sequences only clustered with databased sequences at a level close enough to be considered within the same genus (Table 4.1). Several sequences did not group with any genus and have been indicated as unidentified (Table 4.1).

When sequences obtained in this study were compared with those available in public databases, almost half (148) showed a close match to *Ceratothripoides brunneus* Bagnall

(Table 4.1). This was the most prevalent species in all four areas and completely dominated the thrips community at Bungoma (Table 4.1). There was very low genetic variation with all sequences being more than 99% identical to each other and to Kenyan and South African sequences obtained from GenBank. *T. tabaci* was represented by 47 sequences, and *F. occidentalis* by 50 sequences. These occurred in all areas except Bungoma. The remaining 44 sequences were divided among species that were present only in low numbers. Among the identified thrips species there were four known vectors of Tospoviruses, *F. occidentalis, T. tabaci, F. schultzei,* and *Scirtothrips dorsalis*.

Table 4.1 Numbers of CO1 sequences obtained from thrips species occurring in tomato production systems in four areas in Kenya

Thrips species	**Nakuru**	**Kirinyaga**	**Loitokitok**	**Bungoma**
Ceratothripoides brunneus	32	37	13	66
Thrips tabaci	29	13	5	0
Frankliniella occidentalis	25	12	13	0
Frankliniella 'schultzei'	1	3	2	0
Scirtothrips dorsalis	0	3	3	0
Scirtothrips aurantii	4	0	1	0
Hydatothrips adolfifriderici	0	2	0	0
Haplothrips ganglbaueri	0	1	0	0
Haplothrips sp.	2	6	3	0
Thrips sp.	4	1	3	0
Megalurothrips sp.	1	4	1	0
Frankliniella sp.	0	0	2	1
Unidentified	2	3	3	0

The thrips species were collected from 16 plant species representing eight families. *F. occidentalis* was collected from the highest number of plant species and all eight families (Table 4.2). *C. brunneus* and *T. tabaci* were collected from 12 and 6 plant species, respectively. Solanaceae and Asteraceae were the families with largest number of plant species from which thrips were collected. Brassicaceae family had two plant species which hosted many of the thrips species identified.

Table 4.2 Thrips species reported on different host plants in tomato production areas in Kenya

Thrips species	**Host plants**
Frankliniella occidentalis	*Solanum lycopersicum* L., *Datura stramonium* L. (Solanaceae), *Tithonia diversifolia* (Hemsl.) A. Gray, *Bidens pilosa* L., *Galinsoga parviflora* Cav., *Acanthospermum hispidum* D.C. (Asteraceae), *Amaranthus hybridus* L. (Amaranthaceae), *Brassica oleracea* L. var. *capitata* L., *B. oleracea* L. var. *acephala* D.C. (Brassicaceae), *Phaseolus vurlgaris* L., *Crotalaria incana* L. (Fabaceae), *Richardia brasiliensis* Gomes (Rubiaceae), *Cleome gynandra* L. (Cleomaceae), *Zea mays* L. (Poaceae)
Ceratothripoides brunneus	*S. lycopersicum*, *T. diversifolia*, *A. hybridus*, *D. stramonium*, *B. pilosa*, *B. oleracea* var. *capitata*, *B. oleracea* var. *acephala*, *G. parviflora*, *A. hispidum, P. vurlgaris*, *C. gynandra*, *Z. mays*.
Thrips tabaci	*S. lycopersicum*, *T. diversifolia*, *A. hybridus*, *D. stramonium*, *B. pilosa*, *B. oleracea* var. *acephala*, *Pisum sativa* L. (Fabaceae)
Frankliniella schultzei	*S. lycopersicum*, *T. diversifolia*, *P. vurlgaris*
Scirtothrips dorsalis	*S. lycopersicum*, *A. hybridus*, *D. stramonium*, *B. oleracea* var. *capitata*
Scirtothrips aurantii	*S. lycopersicum*
Megalurothrips sp.	*A. hybridus*
Hydatothrips adolfifriderici	*S. lycopersicum*, *A. hispidum*
Haplothrips ganglbaueri	*S. lycopersicum*
Thrips sp.	*S. lycopersicum*, *T. diversifolia*, *D. stramonium*, *B. oleracea* var. *acephala*, *G. parviflora*
Haplothrips sp.	*S. lycopersicum*, *A. hybridus*, *B. pilosa*, *B. oleracea* var. *acephala*, *G. parviflora*, *R. brasiliensis*, *T. diversifolia*, *D. stramonium*
Frankliniella sp.	*S. lycopersicum*
Unidentified species	*Z. mays*, *B. pilosa, T. diversifolia*, *S. lycopersicum*, *B. oleracea* var. *capitata*, *B. oleracea* var. *acephala*

The phylogenetic analysis indicated that the *T. tabaci* group was genetically diverse with three distinct groups corresponding to the tobacco strain, and the polyphagous thelytokous and arrhenotokous groups (Fig. 4.1). The arrhenotokous group was further divided into two subgroups with 98% bootstrap support. Thrips species collected from tomato production areas clustered with the arrhenotokous group. Most of the Kenyan specimens formed a distinct

subgroup, along with two databased sequences from Japan. Representatives of this subgroup occurred among collections from Nakuru, Kirinyaga and Loitokitok. Some of the specimens from Nakuru and Kirinyaga clustered together with the main subgroup of databased sequences within the arrhenotokous group. Thrips specimens collected from bedding plants in Nairobi (ILRI 1–ILRI 6) grouped with sequences from the thelytokous group.

Most Kenyan specimens in the *Frankliniella* group clustered with databased sequences from *F. occidentalis* (Fig. 4.2). There was a very low level of sequence diversity among specimens of *F. occidentalis* collected in this study, and they were also highly similar to databased sequences from Kenya. All Kenyan sequences belonged to the G group of western flower thrips. Sequences from the databases identified as being from *F. schultzei* formed a diverse group that appeared to be a species complex (Fig. 4.2). Eight Kenyan specimens clustered into one subgroup with some (but not all) sequences of *F. schultzei* from South Africa. Specimen LO58 was identical to a sequence from an unidentified species from North America. Specimens LO34 and BT70 formed a separate cluster that was not closely related to any of the known *Frankliniella* spp. in the databases.

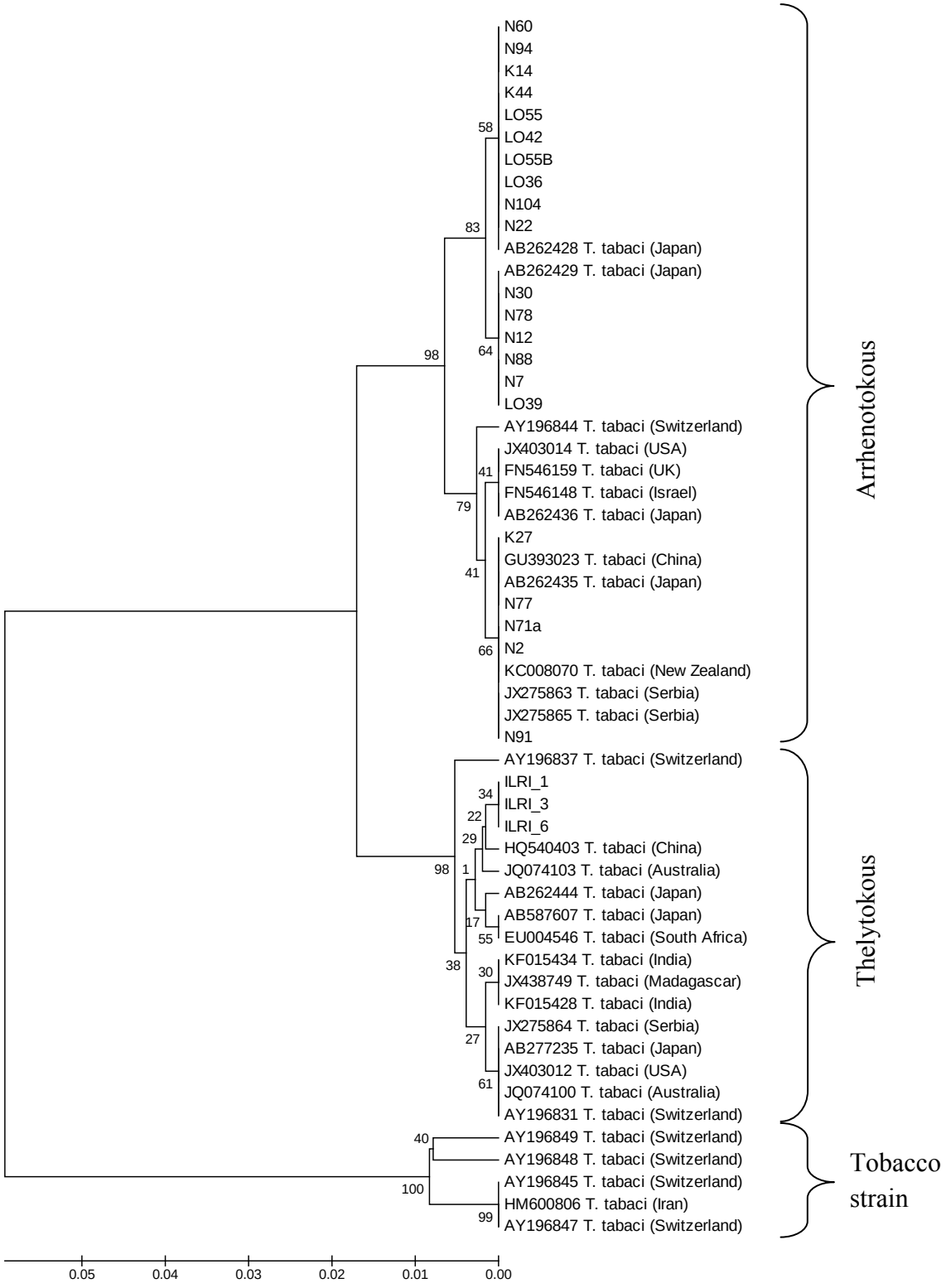

Figure 4.1 Phylogenetic tree of CO1 from specimens of *Thrips tabaci* from tomato production areas in Kenya and databased sequences. Distances were calculated using the Kimura two-parameter method. The tree was drawn using the UPGMA method with 1000 bootstrap replicates in MEGA5. Other phylogenetic analyses gave similar topologies. Kenyan samples are labelled N (Nakuru), K (Kirinyaga) and LO (Loitokitok). Samples labelled ILRI came from ornamentals in Nairobi, Kenya. Only selected sequences are shown in the tree.

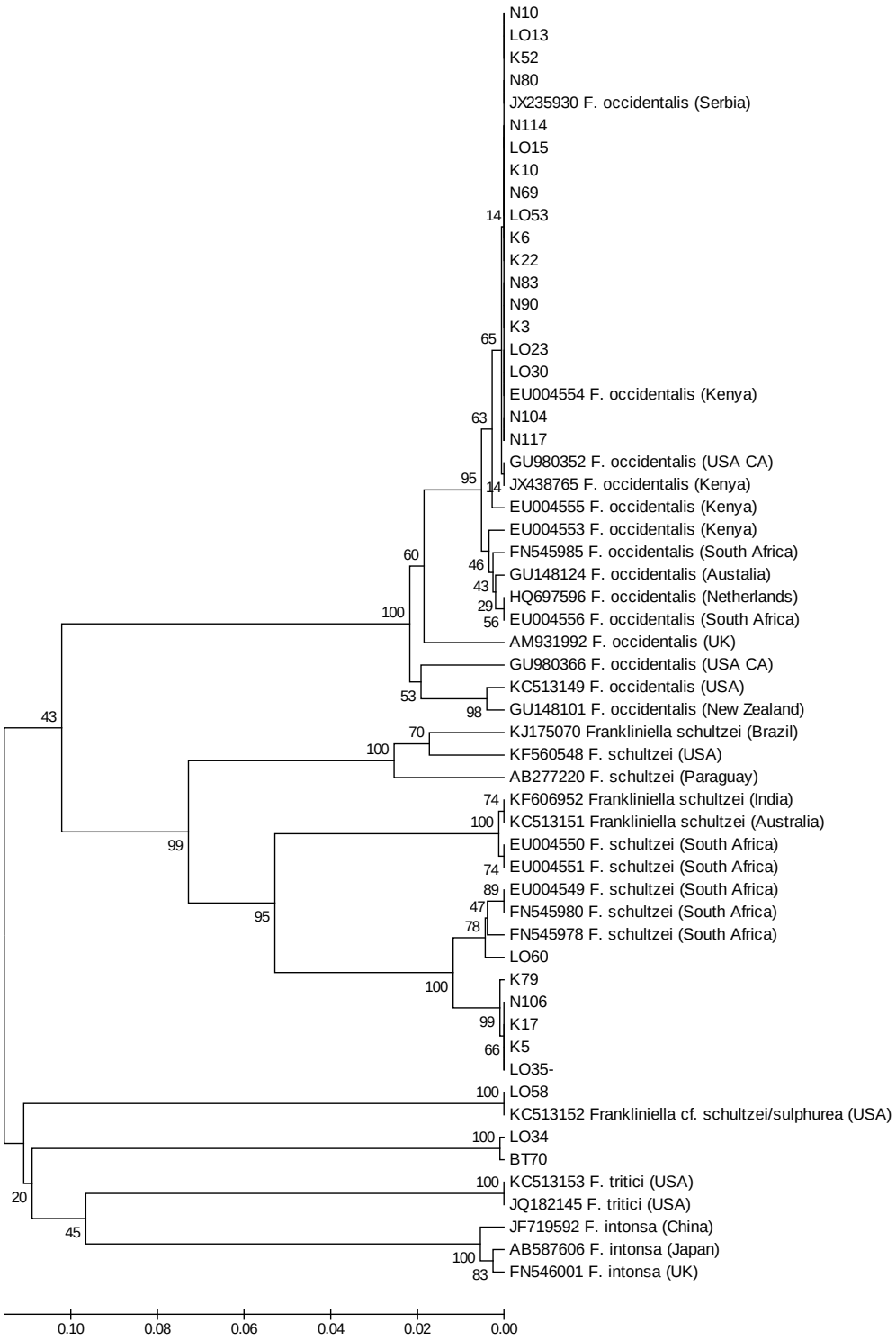

Figure 4.2 Phylogenetic tree of CO1 from specimens of *Frankliniella* species from tomato production areas in Kenya and databased sequences. Distances were calculated using the Kimura two-parameter method. The tree was drawn using the UPGMA method with 1000 bootstrap replicates in MEGA5. Other phylogenetic analyses gave similar topologies. Kenyan samples are labelled N (Nakuru), K (Kirinyaga), LO (Loitokitok) and BT (Bungoma). Only selected sequences are shown in the tree.

Thrips as vectors of *Tomato spotted wilt virus*

A total of 15 out of 58 individual thrips samples analysed tested positive for TSWV using the N protein gene in RT-PCR. The virus was isolated from thrips samples identified as *T. tabaci* and *F. occidentalis*. Selected samples that tested positive were submitted for sequencing to confirm the identity of the virus. The samples yielded sequences which matched with TSWV sequences using BLASTn. Percent nucleotide sequence identity ranged from 97.4 to 99% with sequences from the database and ranged from 98.4 to 100% among sequences of Kenyan isolates (Appendix, Fig. A2).

4.4 Discussion

This study revealed the occurrence of diverse thrips species in four major tomato production areas in Kenya. Among those, *C. brunneus* was found to be the most predominant species, while *F. occidentalis* and *T. tabaci* were the second and the third most abundant thrips species in Nakuru, Kirinyaga and Loitokitok. Furthermore, four thrips species, *F. occidentalis, T. tabaci, F. schultzei* and *S. dorsalis* which have been reported as important vectors of tospoviruses were identified. *F. occidentalis* has been rated as the most efficient vector of TSWV (German *et al.*, 1992), and hence its occurrence in tomato production is an important factor in the epidemiology of TSWV.

Thrips were collected from plants of different taxonomic groups (Amaranthaceae, Asteraceae, Brassicaceae, Cleomaceae, Fabaceae, Poaceae, Rubiaceae and Solanaceae), demonstrating the broad feeding habits of thrips. Most thrips species are polyphagous and have been shown to use a wide array of plants as feeding and reproductive hosts (Northfield *et al.*, 2008). *F. occidentalis* and *T. tabaci* were collected from the highest number of plant species, including

tomato and weeds. Occurrence of weed species that are susceptible to TSWV and support the reproduction of vectors is a critical factor in understanding the epidemiology of TSWV (Northfield *et al.*, 2008; Riley *et al.*, 2011b; Wilson, 1998).

Thrips tabaci sequences formed three distinct groups, with isolates from Kenya clustered into two groups. Brunner *et al.* (2004) reported the formation of three well-supported phylogenetic lineages consisting of two groups associated with leeks and the third with tobacco. These lineages were also suggested by Toda and Murai (2007) and Kobayashi and Eisuke (2012), where three groups consisting of tobacco, arrhenotokous and thelytokous types were observed. Though the Kenya *T. tabaci* isolates clustered with arrhenotokous and thelytokous groups, only the arrhenotokous group was identified from samples collected from the tomato production areas. The specimens belonging to the arrhenotokous group further clustered into two subgroups, where one subgroup consisted of sequences from Kenya and Japan while the second had isolates from Kenya together with isolates from Europe, Israel, Japan, the United State, New Zealand and Serbia. The first subdivision in the arrhenotokous group may indicate the long-standing *T. tabaci* population occurring in Kenya, while the second subgroup could represent recent introductions.

Arrhenotokous and thelytokous populations of *T. tabaci* have been shown to possess different ecological characteristics, which include their relative ability to transmit TSWV, host preferences and resistance to chemicals (Chatzivassiliou *et al.*, 2000; Toda & Morishita, 2009; I. Wijkamp *et al.*, 1995). Thelytokous *T. tabaci* from onion and leeks have been reported as not being able to transmit TSWV (Chatzivassiliou *et al.*, 2002; Wijkamp *et al.*, 1995) while those from ornamentals and wild plants were able to transmit TSWV, although in an inefficient way. Westmore *et al.* (2013), on the other hand, reported effective transmission of

TSWV by thelytokous *T. tabaci* from potato. However, efficient or modest transmission of TSWV by arrhenotokous populations hosted on potato, tomato, onion and leek has been documented (Cabrera-La Rosa & Kennedy, 2007; Chatzivassiliou *et al.*, 2002). Based on our phylogenetic analysis, *T. tabaci* in tomato production areas in Kenya belong to the arrhenotokous group, which is generally considered important in TSWV transmission. The detection of TSWV in individuals of this species in this study highlights the risk from these insects.

Frankliniella occidentalis was the second most abundant thrips species in tomato production in Kenya. Globally, *F. occidentalis* has been reported as the most important insect pest in commercial greenhouse and outdoor crops (Kirk & Terry, 2003). In the present study, *F. occidentalis* formed two phylogenetically independent groups, indicating its high diversity. This is consistent with the high polymorphism and genetic variation observed in this species previously. The species comprises a complex of two cryptic species, identified as western flower thrips G (WFTG) and western flower thrips L (WFTL) groups (Rugman-Jones *et al.*, 2010). The Kenyan population grouped with the WFTG group. The glasshouse strain (WFTG) is more established in Europe, Africa and Asia while the 'Lupin strain' (WFTL) has been reported mainly in New Zealand and California (Rugman-Jones *et al.*, 2010). Moreover, the two groups of *F. occidentalis* differ with the 'glasshouse strain' being widely distributed and more resistant to chemicals (Brødsgaard, 1994; Jensen, 2000). Given its importance in TSWV transmission, detection of virus in individuals of this species, and the diversity in distribution, *F. occidentalis* should be regarded as an important thrips species in the management of TSWV disease in Kenya.

Databased sequences identified as *F. schultzei* clustered into three groups that were distinctive enough to represent different species. Kenyan specimens from this study clustered with sequences from the North West Province of South Africa, and were distinct from one group of sequences from the Americas, and a second group from the Western Cape Province of South Africa, India and Australia. The Kenyan specimens may belong to an undescribed species within the *F.schultzei* complex that is widespread in southern and eastern Africa. *F. schultzei* was reported as a more efficient vector of *Groundnut ringspot virus* than *F. occidentalis,* but consistently had low transmission levels for TSWV (de Borbón & Gracia, 1996; de Borbón *et al.*, 2006). Although *F. schultzei* exists in two colour forms, a dark form and a pale form (Sakimura 1969), because these have been placed in a single polymorphic species since there are no other morphological differences. The dark form of *F. schultzei* is considered to be a more efficient vector of tospoviruses than the pale form (de Borbón & Gracia, 1996; de Borbón *et al.*, 2006; Sakimura, 1969; Sakurai, 2004; Wang *et al.*, 2010; Wijkamp *et al.*, 1995). However, thrips referred to as *F. schultzei* clearly belong to a complex of species and it is not apparent which entity was used in most experiments on virus transmission. There is a clear need for rigorous work on the systematics of this group. *F. schultze*i-like thrips were much less common in this survey than *T. tabaci* and *F. occidentalis*, and are likely to play only a small role in the epidemiology of TSWV in Kenya.

Ceratothripoides brunneus was found to be the predominant species in all the tomato production areas. It occurred mainly on tomatoes but was also reported on 11 other plant species. *C. brunneus* is a species reported as native to Africa but it has recently been found in three other continents: Asia (Peninsula Malaysia), Europe (the Netherlands) and South America (Puerto Rico) (Mound & Azidah, 2009; Mound & Nickle, 2009). In Malaysia, *C. brunneus* was reported for the first time as a predominant thrips species in the lowlands

(Mound & Azidah, 2009). Very little is known about the biology of *C. brunneus*, including its ability to transmit tospoviruses. The related species, *Ceratothripoides Claratris* (Shumsher), which is native to Asia and has been reported as transmitting the *Tospovirus*, *Capsicum chlorotic virus,* in tomatoes in Thailand (Premachandra *et al.*, 2005; Steenken & Halawel, 2011). In a parallel survey (Macharia, *et al.*, 2015a,b), we were unable to demonstrate the presence of TSWV at Bungoma, where *T. tabaci* and *F. occidentalis* could not be detected, but *C. brunneus* was abundant. This suggests that *C. brunneus* is not a vector of the virus. However, this species is very widespread and abundant in tomato production areas, and the ability of other species in the genus to transmit tospoviruses means that it should be investigated in more detail for its risk as a vector of tomato viruses.

Samples of *F. occidentalis* and *T. tabaci* infected with TSWV were observed in Nakuru, one of the tomato production areas where large samples of tomato infected with TSWV were identified, indicating their role in the transmission of the virus. The incidence of viruliferous thrips has been shown to be an important factor contributing to TSWV outbreaks (Okazaki *et al.*, 2007). This has been shown to be particularly important for viruliferous thrips migrating from outside the field since they act as a primary source of inoculum for TSWV early in the production season (Rotenberg *et al.*, 2009). Therefore the management focus to control TSWV in the field should be on *F. occidentalis* and *T. tabaci*.

This study has demonstrated the occurrence of diverse thrips species in tomato production areas in Kenya. Among the identified thrips species were important vectors of TSWV, i.e. *F. occidentalis, T. tabaci* and *F. schultzei*. This study provides useful information for the control of TSWV by the identification of these thrips species as the vectors of the virus. Further, as the thrips were collected from a wide range of plant species occurring in the four tomato

production areas, including weeds and cultivated crops, future investigations into the ability of these plants to support thrips reproduction and susceptibility to TSWV will be necessary in order to establish their role in TSWV epidemiology.

Chapter 5. Weed species in tomato production and their role as alternate hosts of Tomato spotted wilt virus and its vector *Frankliniella occidentalis*

5.1 Introduction

Tomato spotted wilt virus (TSWV) (genus *Tospovirus*, family *Bunyaviridae*) is one of the most devastating plant viruses. It infects and causes disease on many economically important plant species including vegetables and ornamentals (German *et al.*, 1992; Hanssen *et al.*, 2010; Scholthof *et al.*, 2011). It is transmitted by at least nine species of thrips in a persistent and propagative manner (Riley *et al.*, 2011b), of which *Frankliniella occidentalis* (Pergande) is considered to be the most important vector (German *et al.*, 1992). The virus has a unique relationship with the vectors where transmission occurs only when it is acquired during the larval stage (Wijkamp *et al.*, 1993), with no transmission when adults are fed on infected plant materials. The virus has been shown to replicate in the insect body, and infected adult thrips remain viruliferous throughout their life (Assis Filho *et al.*, 2002; Kritzman *et al.*, 2002; Nagata *et al.*, 2002; Whitfield *et al.*, 2005).

The virus infects a wide range of host plants comprising over 1090 plant species in over 84 plant families (Parrella *et al.*, 2003). Its wide host range increases the difficulty of managing the virus. The virus is not transmitted through seeds and there is no record of trans-ovarian transmission, which indicates that each generation of thrips must acquire the virus for new infection to occur (van de Wetering, 1999; Wijkamp *et al.*, 1995). Therefore, host plants should support thrips vector populations for at least a generation to function as a source of TSWV inoculum. In most of the East African countries tomato production is mainly practised

during the dry period to avoid foliar diseases (Macharia, *et al.*, 2015a; Masinde *et al.*, 2011; Ssekyewa, 2006). The virus and vector must therefore survive in alternate hosts during the tomato free production period.

Weeds have been reported as important alternate hosts of tospoviruses and have been shown to act as reservoirs of the virus between cropping seasons (Gracia *et al.*, 1999; Parrella *et al.*, 2003). Weeds that are susceptible to both tospoviruses and thrips have also been shown to be important in the introduction and spread of the virus (Groves *et al.*, 2002; Northfield *et al.*, 2008). The attractiveness of weeds to thrips, their suitability for thrips reproduction, the period in which they bloom, and their life span have been reported as important factors in disease epidemiology (Chatzivassiliou *et al.*, 2001). Plants susceptible to the virus but that do not support thrips reproduction have been shown to be a dead end in virus spread (Duffus, 1971). Therefore, weeds are probably the main reservoir of the virus but their potential contribution to epidemics depends on the number of infected plants as well as their level of infestation by thrips.

Several studies on the importance of weeds as reservoirs of TSWW and as hosts of different thrips species have been undertaken in temperate production systems in Europe, North and South America, Asia and Australia (Atakan *et al.*, 2013; Chatzivassiliou *et al.*, 2007; Gracia *et al.*, 1999; Groves *et al.*, 2002; Okazaki *et al.*, 2007; Wilson, 1998). Few similar studies have been carried-out in tropical areas. Differences in environment will lead to differences in weed flora and biology, which will result in differences in their interactions with thrips and TSWV. There have been no studies carried out in eastern Africa to establish the occurrence of weeds in tomato production systems and their role in TSWV epidemiology. The main aim of this study was to establish the weed species occurring in tomato production in Kenya and

their significance as alternate hosts of TSWV and its main vector *Frankliniella occidentalis* with a view to understanding their role in disease epidemiology.

5.2 Materials and Methods

5.2.1 Survey of weed species in tomato production

A survey was carried out to establish the range of weeds occurring in four major tomato production areas in Kenya; Kirinyaga (Kirinyaga county, 0.64° S, 37.35° E) and Nakuru (Nakuru county, 0.25° S, 36.1° E) were surveyed in March 2013, and Loitokitok (Kajiado county, 2.9° S, 37.5° E), and Bungoma (Bungoma county, 0.6° N, 34.6° E) in June–July 2013. Tomato farms were selected randomly within the four production areas. Weeds occurring in the tomato fields in each farm were sampled using five random quadrats measuring 1 m × 1 m. Weed species were identified, counted and their growth stage recorded. A total of 88 farms were surveyed at Kirinyaga (24), Nakuru (29), Loitokitok (14) and Bungoma (21).

5.2.2 Transmission of TSWV through mechanical inoculation

A greenhouse assay was done to determine the reaction of selected weed species to TSWV infection using mechanical inoculation. The weeds used in the study were selected based on their abundance, family, life form and growth characteristics. Because the experiments were conducted at the University of New England in Australia, seeds were collected from natural infestations in north eastern New South Wales (Table 5.1). Collected weeds consisted of 13 common species that had been observed in Kenya and an additional three weed species (*Bidens subalternans, Datura ferox* and *Solanum chenopodioides*) resembling and easily confused with some of the weeds observed in Kenya. *Commelina benghalensis* could not be

obtained so *C. cyanea* was substituted in the experiments. Two tomato varieties, Moneymaker as susceptible and Swanson as resistant were used as controls. The seeds were pre-treated with either cold treatment or mechanical scarification or both before they were established in the greenhouse in trays containing potting mixture. The seedlings were transferred into 2 kg pots with soil after attaining 2–4 true leaves depending on weed species. After establishment, weeds were mechanically inoculated with TSWV using sap from *Datura stramonium* that had been inoculated with an aggressive strain of TSWV from peanut. Leaf tissue was extracted in 0.1 M potassium phosphate buffer, pH 7.2, containing 1% sodium sulphite and 1% carborundum powder and the extract rubbed onto all but the youngest leaves of each plant. All plants were kept for 24 h in the dark before and after inoculation to enhance their susceptibility to the virus. Inoculation was repeated 3 times to ensure effective infection and to avoid escapes. The interval between inoculations ranged from 2–5 days depending on the damage caused to the plants. The weeds were maintained in an insect proof greenhouse (temperature 25±2 °C) and observed for symptom expression. The treatments were replicated in 10 plants in a completely randomised design. Data on incidence were collected at least 30 days after inoculation depending on growth rate and symptom development. Leaf samples were assayed using a DAS-ELISA kit (Agdia, Elkhart, IN, USA) where ELISA readings were presumed to indicate virus concentration.

For DAS-ELISA, sap was extracted by grinding leaf samples in general extraction buffer provided in the kit at a ratio of 1:10 (w/v) and 100 µl of the sample was loaded into a well in a microtitre plate coated with TSWV specific antibody. Positive and negative controls were also included. The plate was incubated overnight at 4°C after which the plate was washed with phosphate buffered saline-Tween 20 (PBS-T) buffer. The plates were further processed according to the manufacturer's protocol. Absorbance (A_{405}) was recorded after one hour

incubation using a microplate reader (Epoch, BioTEK, VT, USA). Samples with absorbance equal to or greater than 3 times the average negative control were considered positive.

Table 5.1. Weed species used in experiments and their abundance in the field

Family	Botanical name	Abundance[1]
Amaranthaceae	*Amaranthus hybridus*	High
Asteraceae	*Bidens pilosa*	High
	Bidens subalternans	-[2]
	Conyza canadensis	Low
	Galinsoga parviflora	High
	Sonchus oleraceus	Medium
	Tagetes minuta	High
Chenopodiaceae	*Chenopodium album*	Medium
Commelinaceae	*Commelina cyanea*	-
Fabaceae	*Trifolium repens*	Medium
Malvacea	*Malva parviflora*	Low
Oxalidaceae	*Oxalis latifolia*	High
Potulacaceae	*Portulaca oleracea*	Low
Solanaceae	*Datura ferox*	-
	Datura stramonium	High
	Solanum chenopodioides	-
	Solanum nigrum	High

[1]Relative abundance of the species in surveys in Kenya.

[2]Species not recorded during survey. *Commelina benghalensis* had high abundance.

5.2.3 Laboratory culture of *F. occidentalis*

F. occidentalis was sourced from Biological Services, Loxton, South Australia through Prof. Graham Hall. The identity of the thrips was confirmed using an online morphological key (L. A. Mound *et al.*, 2014). The thrips were reared on cucumber using a modified rearing

technique based on DeGraaf and Wood (2009). Cucumbers were placed into 2 L glass jars with lids covered with 150 µm mesh. The culture was maintained at 25ºC with 16:8 hours light and dark. The rearing was synchronised with cucumbers being changed after every 3 days into new jars to allow hatching and larval development leading to different stages of thrips that were used in different experiments. The jars were cleaned after every 3 weeks.

Viruliferous thrips were established by feeding freshly emerged first larval instars with tomato leaves infected with TSWV. The larvae were allowed an acquisition period of 24–48 hours. The larvae were then reared to maturity and the resulting adult thrips were maintained on cucumbers in glass jars.

Quantitative reverse transcription-PCR (qRT-PCR) was used to identify and quantify TSWV in individual thrips (Rotenberg *et al.*, 2009). Total RNA was extracted from individual insects using the method described by Boonham *et al.* (2002) by homogenising in nuclease-free water and mixing with 50 µl of a 50% (w/v) slurry of Chelex 100 (Bio-Rad). The samples were heated at 94°C for 5 min and centrifuged for 5 min at 13000 *g* at 4°C. The supernatant was used as a template in qRT-PCR. The *F. occidentalis* actin primers described by Boonham *et al.* (2002) (forward primer 5′-GGT ATC GTC CTG GAC TCT GGT G-3′ and reverse primer 5′-GGG AAG GGC GTA ACCT TCA-3′) were used to amplify the actin gene as an internal reference and TSWV nucleocapsid (N) gene primers described by Whitfield *et al.* (2008) (forward primer 5′-GCT TCC CAC CCT TT GAT TC-3′ and reverse primer 5′-ATA GCC AAG ACA ACA CTG ATC-3′) were used for the virus. The one step reaction mix consisted of 1–2 µl of total RNA extract, 10 µl SensiFAST™ SYBR® no-ROX one step mix (Bioline, Sydney, Australia), 200 nM of each primer, 0.2 µl reverse transcriptase and 0.4 µl RiboSafe RNase Inhibitor in a final volume of 20 µl. Reverse transcription was performed at 45°C for

10 min followed by DNA denaturation at 95°C for 2 min and 40 amplification cycles of 95°C for 5 s and 60°C for 20 s in a Rotorgene 6000 real-time PCR machine (Qiagen, Germany). Melting curve analysis was done after the final PCR cycle by increasing the temperature by 0.5°C per min from 60°C to 95°C. The abundance of TSWV-N RNA was calculated using the inverse equation of (Pfaffl, 2001): $E_{actin}^{Ct(actin)}/E_N^{Ct(N)}$; where E = PCR efficiency of a primer pair (actin or N), and Ct = the amplification cycle.

5.2.4 Transmission of TSWV using *F. occidentalis*

The potential of *F.occidentalis* to transmit TSWV from tomatoes to selected weed species was evaluated. Weeds were established in the greenhouse as described above and after attaining 3–5 true leaves, 6 plants of each species were transferred into thrips-proof cages measuring 60 × 60 × 60 cm (BugDorm, Taichung, Taiwan) and infested with 10 viruliferous *F. occidentalis* adults per plant raised from larvae on infected tomato leaves as described earlier. Virus titre in thrips was evaluated in samples collected before inoculation using qRT-PCR. Weeds were maintained in the greenhouse and observed for symptom development. The feeding damage from thrips infestation was evaluated on a scale of 0–3 (Maharijaya *et al.*, 2011) while the reproduction potential was evaluated qualitatively by tapping the plants over a white surface and observing the relative abundance of larvae. The plants were assayed for TSWV infection three weeks after infestation using DAS-ELISA kits (Agdia, USA) and the infection was confirmed using reverse transcription PCR (RT-PCR).

RNA was extracted from weed samples using Trizol reagent (Life Technologies, Mulgrave, VIC, Australia) according to the manufacturer's protocol. Leaf tissue (100 mg) was ground to a fine powder in liquid nitrogen and 1 mL of Trizol reagent was added to each tube and vortexed thoroughly, then incubated for 5 min at room temperature to allow complete

dissociation of nucleoprotein complexes. Chloroform was added into the mixture at the rate of 200 µl per 1 ml of Trizol used, shaken vigorously and incubated for 3–5 minutes at room temperature. The tube was centrifuged at 13000 *g* for 15 min at 4°C and the upper aqueous phase transferred into a fresh 1.5 ml sterile tube. RNA was precipitated by adding 500 µl of ice-cold isopropanol and the tube incubated for 10 min at room temperature. The tubes were centrifuged at 13000 *g* for 10 min. The supernatant was discarded and the pellet washed with 500 µl of 75% (v/v) ethanol. After drying, the pellet was resuspended in 50 µl of RNase-free water and stored at −80^{o}C.

Reverse transcription-PCR (RT-PCR) was performed using two primer sets: TSWV 722 (5'-GCT GGA GCT AAG TAT AGC AGC-3') and TSWV 723 (5'-CAC AAG GCA AAG ACC TTG AG-3') (Adkins & Rosskopf, 2002); and TSW 1 (5'-TCT GGT AGC ATT CAA CTT CAA-3') and TSW 2 (5'-GTT TCA CTG TAA TGT TCC ATA G-3') (Roberts *et al.*, 2000). One step RT-PCR was carried out using the QIAGEN OneStep RT-PCR Kit (Qiagen, Chadstone, VIC, Australia) with 1 µl of RNA extract in a 25 µl reaction mix of 5 µl of 5× one step PCR buffer (containing 2.5 mM $MgCl_2$), 400 µM dNTP mix, 0.4 µM of forward and reverse primers and 1 µl of one step RT-PCR enzyme mix (containing reverse transcriptase and Hotstar Taq DNA polymerase). The reactions for TSW 1 and 2 were performed as follows: reverse transcription at 48°C for 45 min; 95°C for 15 min to activate the Hotstar Taq DNA polymerase and inactivate reverse transcriptase; 40 cycles of 94°C for 45 s denaturation of cDNA, 45°C for 45 s annealing and 72°C for 1 min extension; and a final extension at 72°C for 10 min. The reactions for TSWV 722 and 723 were similar except that annealing was done at 55 ^{o}C for 45 s, and 35 cycles of amplification performed. Three µl of resulting PCR products were mixed with 6 µl of orange G loading buffer (150 mg/mL Ficoll 400, 2.5 mg/mL Orange G) containing 1:200 (v:v) GelStar™ Nucleic Acid Gel Stain (Lonza,

Rockland, ME, USA) and visualised in a 1.5% agarose gel. The expected sizes were 620 bp (TSWV 722 and 723) and 628 bp (TSW1 and 2).

5.2.5 Evaluation of thrips reproduction on detached leaves

Selected weed species and the tomato varieties Grosse Lisse and Moneymaker (TSWV-susceptible) and Klass and Swanson (TSWV-resistant) were evaluated for their ability to support reproduction of *F. occidentalis* using a detached leaf assay. The detached leaves were placed in a 9 cm diameter Petri dish with a window in the lid covered with 150 µm mesh containing 1% water agar amended with 0.01% Terraclor fungicide (750 mg/g pentachloronitrobenzene) to prevent fungus growth. Five adult thrips were introduced onto each leaf and the Petri dish sealed with Parafilm to prevent the thrips from escaping. Thrips were allowed to feed and oviposit for three days under 16:8h light/dark, at temperature of 25ºC and 50–60% relative humidity. After three days, adult thrips were removed and number of 1st and 2nd larvae instar and feeding punctures/damage was recorded. The feeding or damage was assessed using a relative scale from 0 (no damage) to 3 (severe damage) (Maharijaya *et al.*, 2011). The Petri dishes were re-sealed with Parafilm and incubated as above for an additional three days. The 1st and 2nd larvae instar larvae that emerged from each leaf in each weed species were counted.

5.3 Results

5.3.1 Survey of weed species in tomato production

A total of 43 species of weeds representing 19 families were found in the tomato production areas (Table 5.2). The family Asteraceae had the highest (9) number of species while Solanaceae and Poaceae had six and five species, respectively (Table 5.2, Appendix, Table

A3). Most of the weeds (29 species) have previously been recorded as hosts of TSWV, while two species (*Ricinus communis* and *Digitaria scalarum*) have been reported as non-hosts (Table 5.2). Eleven species, including the relatively abundant *Commelina benghalensis* and *Oxalis latifolia*, were of unknown host status. The majority of the weed species identified were annual (27 species), 12 were perennial and the remainder could be either annual or short-lived perennial (Table 5.2).

Bidens pilosa, Amaranthus hybridus, Galinsoga parviflora and *Commelina benghalensis* were the most frequently found weed species across the four production areas (Table 5.3). The highest number of weed species (34) was found in the Kirinyaga area while 25 species were found at each of Nakuru and Bungoma and 23 species at Loitokitok. Among the observed weeds, 21 occurred in at least three of the production areas while 16 occurred in only one of the production areas. Most of these were found at low frequency, except *Acanthospermum hispidum* and *Richardia brasiliensis,* which were found on 58% and 54% respectively, of the farms at Kirinyaga (Table 5.3).

Table 5.2 Weeds identified in tomato production areas in Kenya, biological type and their status as hosts of *Tomato spotted wilt virus*

Family	Botanical name	Biological type	Host of TSWV	Reference
Amaranthaceae	*Amaranthus hybridus*	Annual	Yes	(Cho *et al.*, 1986)
	Amaranthus spinosus	Annual	Yes	(Cho *et al.*, 1986)
	Amaranthus retroflexus	Annual	Yes	(Stobbs *et al.*, 1992)
Asteraceae	*Acanthospermum hispidum*	Annual	Yes	(Parrella *et al.*, 2003)
	Achyranthes aspera	Perennial	?	No record
	Bidens pilosa	Annual	Yes	(Cho *et al.*, 1986),
	Galinsoga parviflora	Annual	Yes	(Cho *et al.*, 1986)
	Lactuca serriola	Annual or biennial	Yes	(Stobbs *et al.*, 1992)
	Senecio vulgaris	Annual	Yes	(Stobbs *et al.*, 1992)
	Sonchus oleraceus	Annual	Yes	(Cho *et al.*, 1986)
	Tagetes minuta	Annual	Yes	(Helms *et al.*, 1960)
	Tithonia diversifolia	Annual or short-lived perennial	Yes	(Parrella *et al.*, 2003)
Boraginaceae	*Cynoglossum coeruleum*	Perennial	Yes	(Parrella *et al.*, 2003)
Brassicaceae	*Brassica napus*	Annual	Yes	(Parrella *et al.*, 2003)
	Capsella bursa-pastoris	Annual	Yes	(Cho *et al.*, 1986)
Capparaceae	*Cleome gynandra*	Annual	?	No record
Chenopodiaceae	*Chenopodium album*	Annual	Yes	(Cho *et al.*, 1986)
Commelinaceae	*Commelina benghalensis*	Perennial	?	No record
Convolvulaceae	*Ipomoea purpurea*	Annual	Yes	(Parrella *et al.*, 2003)
Euphrbiaceae	*Euphorbia heterophylla*	Annual	Yes	(Johnson *et al.*, 1995)
	Ricinus communis	Perennial	No	(Parrella *et al.*, 2003)
Fabaceae	*Crotalaria polysperma*	Annual	?	No record
	Trifolium repens	Perennial	Yes	(Stobbs *et al.*, 1992)
	Medicago sativa	Perennial	?	No record
Labiatae	*Leonotis nepetifolia*	Annual	?	No record
Malvacea	*Malva parviflora*	Annual or perennial	Yes	(Cho *et al.*, 1986)
Oxalidaceae	*Oxalis latifolia*	Perennial	?	No record
	Oxalis corniculata	Annual or perennial	Yes	(Marchoux and Gebre-Selassiè, 1991)
Poaceae	*Eleusine indica*	Annual	?	No record
	Digitaria scalarum	Perennial	No	(Parrella *et al.*, 2003)
	Cynodon dactylon	Perennial	Yes	(Jordá *et al.*, 1995)
	Setaria verticillata	Annual	?	No record
Polygonaceae	*Oxygonum sinuatum*	Annual	?	No record
	Fallopia convolvulus	Annual	Yes	(Parrella *et al.*, 2003)
Potulacaceae	*Portulaca oleracea*	Annual	Yes	(Cho *et al.*, 1986)
Rubiaceae	*Richardia brasiliensis*	Perennial	Yes	(Jordá *et al.*, 1995)
Solanaceae	*Solanum nigrum*	Annual or short-lived perennial	Yes	(Cho *et al.*, 1986)
	Datura stramonium	Annual	Yes	(Stobbs *et al.*, 1992)
	Nicandra physalodes	Annual	Yes	(Cho *et al.*, 1986)
	Solanum incanum	Perennial	?	No record
	Datura ferox	Annual	Yes	(Cho *et al.*, 1987)
	Physalis angulata	Annual	Yes	(Cho *et al.*, 1986)
Verbenaceae	*Lantana camara*	Perennial	Yes	(Hausbeck *et al.*, 1992)

Table 5.3 Frequency (%) of occurrence of weed species in four tomato production areas in Kenya. Species are ranked in order of overall frequency

Botanical name	Nakuru	Kirinyaga	Loitokitok	Bungoma
Bidens pilosa	89.7	79.2	71.4	90.5
Amaranthus hybridus	69.0	75.0	85.7	76.2
Galinsoga parviflora	89.7	54.2	64.3	95.2
Commelina benghalensis	75.9	91.7	35.7	100.0
Solanum nigrum	69.0	37.5	57.1	71.4
Tagetes minuta	69.0	66.7	42.9	33.3
Datura stramonium	69.0	37.5	28.6	76.2
Sonchus oleraceus	55.2	29.2	71.4	42.9
Nicandra physalodes	65.5	62.5	14.3	33.3
Oxalis latifolia	72.4	16.7	-	85.7
Oxygonum sinuatum	27.6	45.8	21.4	52.4
Malva parviflora	48.3	25.0	35.7	14.3
Cleome gynandra	3.4	16.7	28.6	71.4
Chenopodium album	31.0	12.5	28.6	42.9
Brassica napus	55.2	8.3	28.6	14.3
Portulaca oleracea	24.1	54.2	-	-
Crotalaria polysperma	48.3	4.2	7.1	9.5
Acanthospermum hispidum	-	58.3	-	-
Richardia brasiliensis	-	54.2	-	-
Fallopia convolvulus	34.5	-	14.3	-
Leonotis nepetifolia	10.3	-	35.7	-
Solanum incanum	3.4	16.7	14.3	4.8
Lactuca serriola	3.4	25.0	-	9.5
Eleusine indica	10.3	12.5	-	-
Lantana camara	-	16.7	-	4.8
Senecio vulgaris	-	4.2	7.1	9.5
Digitaria scalarum	3.4	4.2	7.1	4.8
Oxalis corniculata	13.8	-	-	4.8
Euphorbia heterophylla	-	16.7	-	-
Ipomoea purpurea	6.9	4.2	-	4.8
Capsella bursa-pastoris	-	-	14.3	-
Physalis angulata	-	-	14.3	-
Ricinus communis	-	-	14.3	-
Cynodon dactylon	-	12.5	-	-
Amaranthus spinosus	-	12.5	-	-
Cynoglossum coeruleum	-	12.5	-	-
Trifolium repens	-	-	-	4.8
Achyranthes aspera	-	-	-	4.8
Tithonia diversifolia	-	4.2	-	-
Amaranthus retroflexus	-	4.2	-	-
Datura ferox	-	4.2	-	-
Medicago sativa	-	4.2	-	-
Setaria verticillata	-	4.2	-	-

5.3.2 Transmission of TSWV through mechanical inoculation

Twelve out of the 17 weed species that were evaluated became infected with TSWV after mechanical inoculation (Table 5.4). Infected weeds produced a wide range of characteristic symptoms with mosaic and stunting being the most common symptoms. Some of the plants that were positive in the ELISA test did not produce any symptoms. This was true for all infected plants of *Malva parviflora*, but also for individual plants of several other species. *Chenopodium album* produced necrotic local spots and the virus was only identified from the inoculated leaves. Absorbance in the ELISA test for these leaves was just above the threshold for a positive result. Incidence varied among the weed species with the highest disease incidence being recorded on *Datura stramonium, D. ferox* and *C. album*. *Amaranthus hybridus* and *G. parviflora* had the highest virus titre equivalent recorded. All weeds in the Solanaceae family had high virus incidence and a high virus titre ranging from 2.8 to 3.2, which was comparable with the susceptible tomato Moneymaker (3.0) used as the control (Table 5.4). No infections were observed on *Bidens pilosa, Trifolium repens*, *Commelina cyanea, O. latifolia*, *Tagetes minuta* or the resistant tomato Swanson after mechanical inoculation.

Table 5.4 Reaction of selected weed species to *Tomato spotted wilt virus* infection through mechanically inoculation

Botanical name	**Symptoms** [a]	**Incidence**[b]	**ELISA Average**[c]
Amaranthus hybridus	ST, M, N, LD, C	4/9	3.6
Bidens pilosa	-	0/9	-
Bidens subalternans	M, VC	3/9	2.7
Chenopodium album (inoculated leaves)	NS	9/9	0.3
Chenopodium album (uninoculated leaves)	-	0/9	-
Commelina cyanea	-	0/9	-
Datura ferox	LD, M, ST, Mo	9/9	2.8
Datura stramonium	LD, M, ST, Mo	9/9	3.2
Galinsoga parviflora	LD, M, ST	4/9	3.7
Malva parviflora	-	5/9	1.3
Oxalis latifolia	-	0/9	-
Portulaca oleracea	LD	2/9	2.8
Solanum chenopodioides	CR, M, CV, C, ST, LD	7/9	2.9
Solanum nigrum	N, C, M, P, CR, LD	8/9	2.9
Sonchus oleraceus	M, N, C, VC	5/9	2.8
Tagetes minuta	-	0/9	-
Trifolium repens	-	0/9	-
Moneymaker	ST, M, LD, P	9/9	3.0
Swanson	-	0/9	-
STD			0.952

[a] NR-Necrotic ringspot, NS-Necrotic spot, M-Mosaic, Mo-Mottle, LD-leaf deformation, ST-stunting, P- Purpling, C-Chlorosis, (-) no symptom

[b] Number of plants positive by ELISA over number of plants tested.

[c] ELISA average is based on the absorbance for the positive samples. Absorbance of negative controls and plants recorded as negative was ≤ 0.1.

5.3.3 Transmission of TSWV using *F. occidentalis*

Potentially viruliferous *F. occidentalis* successfully transmitted TSWV from infected tomato leaves to many of the weed species (Table 5.5). Quantitative RT-PCR confirmed that *F. occidentalis* had acquired the virus. Relative TSWV titre in individual thrips samples ranged from a normalised value of 0.015 to 283.4 relative to actin gene expression, while the normalised value for the healthy control was 0.0015. The rate of acquisition of TSWV by *F. occidentalis* was 45% of 75 individuals tested.

Bidens pilosa, C. cyanea, C. album, D. stramonium, B. subalternans, T. repens and T. minuta produced characteristic symptoms ranging from mosaic, necrotic local lesions, necrotic spots, vein clearing, stunting and leaf distortion (Table 5.5). Some of the other infected weed species did not produce any symptoms. TSWV infection of weeds was identified using DAS-ELISA and RT-PCR tests (Table 5.5). The highest incidence of infection was observed on *A. hybridus*, *O. latifolia*, *S. chenopodioides, C. album* and *C. cyanea,* while no infection was detected on *G. parviflora* and *Sonchus oleraceus*. The experiment was repeated for these species, again with negative results.

A. hydridus, B. pilosa, G. parviflora, M. parviflora, S. chenopodioides, T. repens and T. minuta supported high survival and reproduction of thrips based on the number of larvae recovered after the experiment (Table 5.5). Weed species that supported high survival and reproduction of thrips also showed high levels of feeding damage.

Table 5.5 Transmission of *Tomato spotted wilt virus* by *F. occidentalis* from tomatoes to selected weed species

Botanical name	**Damage**	**Larvae population [a]**	**Adult at end [b]**	**Symptoms [c]**	**ELISA**	**PCR 722[d]**	**PCR TSW[e] 1**
Amaranthus hybridus	2.8	H	H	–	1/6	6/6	6/6
Bidens pilosa	3.0	H	H	M, VC	1/6	1/6	1/6
Bidens pilosa[f]	2.8	H	H	–	0/6	0/6	0/6
Bidens subalternans	2.5	M	M	M	2/6	1/6	1/6
Chenopodium album	1.0	L	L	NL	0/6	6/6	6/6
Commelina cyanea	1.3	L	L	M	2/6	5/6	4/6
Datura stramonium	1.5	M	M	M	2/6	1/6	1/6
Galinsoga parviflora	1.0	M	H	–	0/6	0/6	0/6
Galinsoga parviflora[f]	2.0	M	M	–	0/6	0/6	0/6
Malva parviflora	2.7	H	H	–	0/6	3/6	2/6
Oxalis latifolia	1.5	L	L	NS	0/6	6/6	6/6
Portulaca oleracea	1.2	M-L	L	–	1/6	3/6	3/6
Solanum chenopodioides	2.5	H	H	–	2/6	6/6	6/6
Solanum nigrum	1.5	M-H	M	–	0/6	3/6	2/6
Sonchus oleraceus	1.0	L	L	–	0/6	0/6	0/6
Sonchus oleraceus[f]	1.5	L	L	–	0/6	0/6	0/6
Tagetes minuta	2.2	H	H	ST, LD	3/6	4/6	4/6
Trifolium repens	2.3	H	H	M, NS	1/6	1/6	1/6
Moneymaker	2	H	M	M, P	1/3	3/3	3/3
Swanson	1	M	M	–	0/3	0/3	0/3

[a, b] L= Low, M= Medium, H= High, M-H= Medium to high, M-L= Low to medium

[c] Symptoms: M = Mosaic, VC = Vein clearing, NL = Necrotic lesion, NS = Necrotic spots, P = Purpling, ST = Stunting, LD = Leaf deformation, – = No symptom

[d, e] PCR was performed using primers TSWV 722 & 723 (Adkins & Rosskopf, 2002) and TSW1 and 2 (Roberts *et al.*, 2000)

[f] Result of experiments that were repeated

5.3.4 Evaluation of thrips reproduction on detached leaves

Selected weed species were found to be suitable feeding hosts and supported reproduction of *F. occidentalis*. Feeding damage was significantly different (Kruskal-Wallis test $\chi^2 = 102.2$, df = 20, $P < 0.001$) among weed species. The highest damage was observed on *A. hybridus* (2.5) and the lowest damage was observed on *P. oleracea* (0.3) (Figure 5.1a). The TSWV-susceptible tomato varieties Grosse Lisse and Moneymaker showed a higher level of damage than the resistant varieties Klass and Swanson. Levels of feeding damage in the detached leaf assay (Figure 5.1a) were consistent with those observed in the greenhouse experiment (Table 5.5).

Reproduction of *F. occidentalis* was significantly different between weed species (Kruskal-Wallis $\chi^2 = 116.68$, df = 20, $P < 0.001$). The highest numbers of larvae were seen on *B. subalternans* whereas *S. oleraceus, C. album* and *P. oleracea* had the lowest. Plants with high larval counts were ranked as follows; *B. subalternans* > *T. minuta* > *S. nigrum* > *A. hybridus* > *S. chenopodioides* > *B. pilosa* > *G. parviflora* > *M. parviflora* ≥ Grosse Lisse > *D. stramonium* = Moneymaker (Fig. 5.1b). TSWV-susceptible tomato cultivars supported more thrips larvae than the resistant cultivars. There was a significant positive correlation ($r = 0.862$, $P < 0.001$) between feeding damage and number of larvae, indicating that thrips oviposited more eggs on hosts they

perceived as suitable. Numbers of larvae counted in the detached leaf assay (Figure 5.1b) were consistent with qualitative assessments of larval numbers in the greenhouse experiment (Table 5.5).

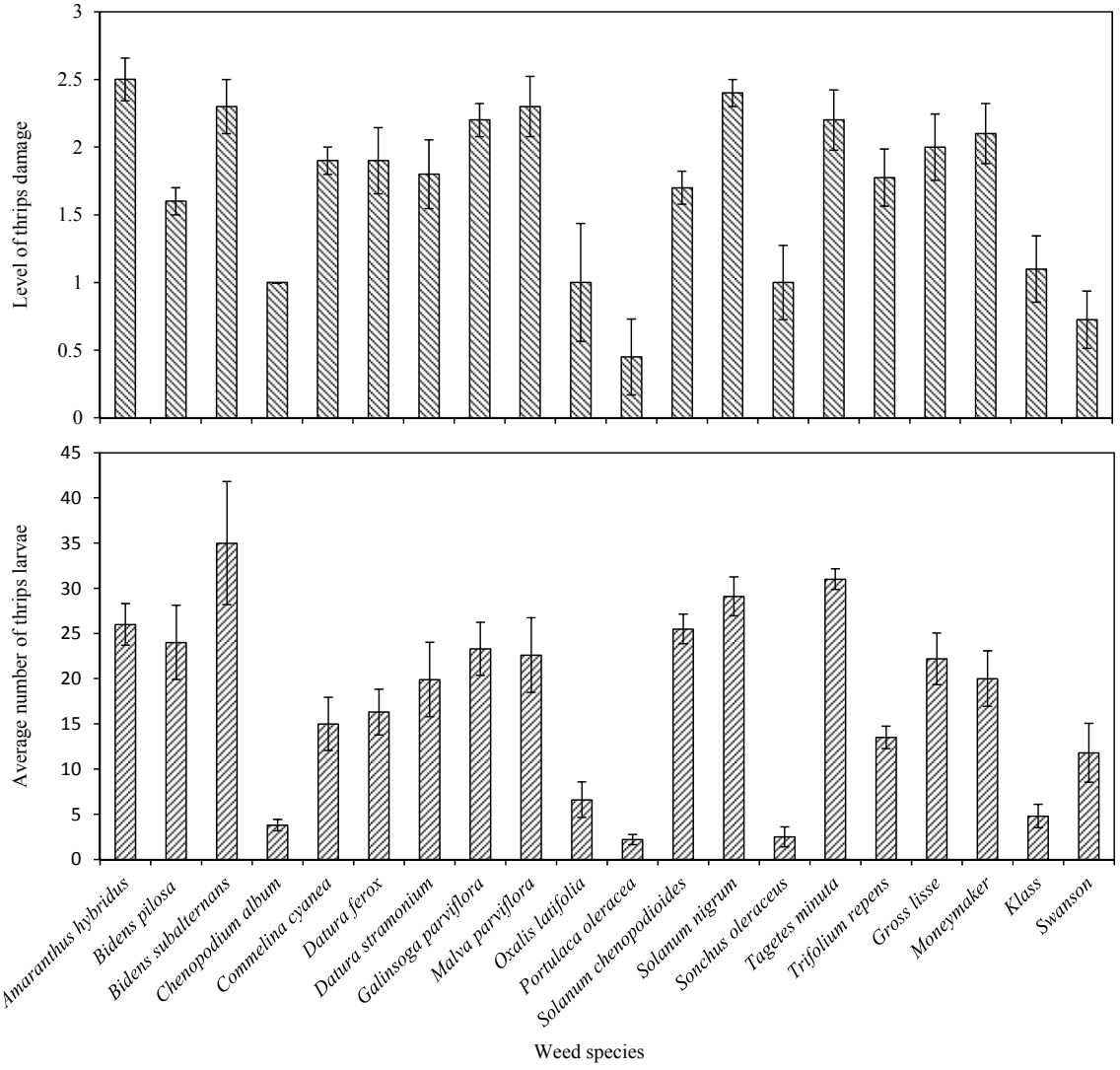

Figure 5.1. a) Level of damage from *F. occidentalis* infestation on weed species and tomato cultivars in a detached leaf assay. b) Average number of larvae of *F. occidentalis* on weeds species and tomato cultivars in a detached leaf assay.

5.4 Discussion

The results revealed occurrence of a wide range of weeds in major tomato production areas in Kenya. The weeds represented 19 plant families. Of these Asteraceae and Solanaceae had the highest number of species recorded and were distributed in all the production areas. Although samples that were collected from the field were not tested for TSWV, most of the weed species that had previously been identified as hosts of the virus were also in the family Asteraceae followed by Solanaceae which is consistent with observations that these families contain large numbers of plants susceptible to TSWV (Parrella *et al.*, 2003). The large number of weeds observed indicates the presence of suitable hosts able to support the virus and its vectors if introduced.

The transmission study resulted in four new hosts of TSWV, *O. latifolia, Bidens subalternans, S. chenopodioides* and *C. cyanea*. The first three species belong to genera with other known hosts. However, no plants in the family Commelinaceae have hitherto been recorded as susceptible to TSWV. Although *C. cyanea* did not occur in the survey area, it is likely that the closely related species *C. benghalensis* would show similar susceptibility to TSWV as *C. cyanea*.

Transmission of TSWV to weeds depends both on their susceptibility to the virus and their suitability as feeding and oviposition hosts of the vector (Kahn *et al.*, 2005). Of the weeds chosen for detailed study, all had at least one plant infected by either mechanical or thrips transmission. Although TSWV was not mechanically transmitted onto *B. pilosa, T. repens, O. latifolia, C. cyanea* or *T. minuta*, there was successful transmission through the vector, *F. occidentalis.* In some cases, such as in *O. latifolia*, infection was only detected by RT-PCR so the apparent failure

of mechanical transmission may have been because DAS-ELISA, which was the only detection method used in the mechanical transmission experiment, was particularly insensitive in this species. Other species may have had anatomical or chemical characteristics that interfered with virus transmission when the plant surface was damaged by carborundum. More interesting are cases where mechanical inoculation was successful, showing susceptibility to the virus, but where infection could not be established by thrips transmission.

The ability of the weeds to support *F. occidentalis* feeding and reproduction was assessed using a detached leaf assay under laboratory conditions and by the use of qualitative data from the greenhouse experiment. Thrips laid high numbers of eggs on plants that they perceived as suitable feeding host, which was evidenced by the high correlation between feeding damage and number of larvae. Thrips have been reported to distinguish the suitability of plants as feeding and oviposition hosts to ensure fitness of their progeny (Nyasani *et al.*, 2013; Scott Brown *et al.*, 2002). The reproduction potential recorded in the detached leaf assay was similar to that observed in the greenhouse experiment. The oviposition and reproductive potential are influenced by the nutritional quality of the host plant and the presence or absence of plant defence compounds (Delphia *et al.*, 2007; Shrestha *et al.*, 2012).

Amaranthus hybridus, D. stramonium and *S. nigrum* had a high frequency in the field and supported high thrips reproduction. Although these species had high transmission rates by mechanical inoculation, there was varied vector transmission with the highest transmission recorded in *A. hydridus*. *Datura stramonium* was found to be a good host for TSWV acquisition by *F. occidentalis* (Bautista *et al.*, 1995), as it had a high virus titre and an even distribution of

infected cells. Furthermore, *D. stramonium* was reported as a good TSWV acquisition and transmission host of *T. tabaci* (Chatzivassiliou *et al.*, 1999; Chatzivassiliou *et al.*, 2007). This reinforces the significance of *D. stramonium* as observed in our current study. However, *A. hybridus,* an annual weed that proliferates fast with several generations per year seem to be a much more important annual host which should also be considered in the management of TSWV.

Bidens pilosa, *G. parviflora* and *S. oleraceus* occurred frequently across the four production areas. All three species have frequently been cited as hosts of TSWV but there is conflicting evidence for their role in transmission of the virus by *F. occidentalis*. Transmission of TSWV to these species by thrips was either very low (*B. pilosa*) or undetectable (*G. parviflora* and *S. oleraceus*) in our experiments, although *G. parviflora* and *S. oleraceus* were readily infected by mechanical transmission. Chatzivassiliou *et al.* (2001) found a high incidence of TSWV infection in *S. oleraceus* in the field in Greece; however, in their later study, were unable to transmit TSWV to *S. oleraceus* using *T. tabaci* due to high larval mortality and poor oviposition preference (Chatzivassiliou *et al.*, 2007). We also found the species to be a poor feeding and oviposition host for *F. occidentalis.* Presumably *S. oleraceus* can become infected when there is a large population of viruliferous thrips that has built up on other species, but it is unlikely to be attractive to thrips if other plants are available nearby.

On the other hand, *B. pilosa* and *G. parviflora* supported a high rate of thrips reproduction but had a low level of TSWV acquisition. There have been reports of high frequency of TSWV infection in field populations of *B. pilosa*, but incidence of infection in *G. parviflora* was low

(Cho *et al.*, 1986). Because these are common weeds in many cropping systems, there is need for further work to establish their importance as reservoirs of the virus.

Portulaca oleracea was shown to be susceptible to TSWV but supported low feeding and reproduction by thrips. Atakan *et al.* (2013) found a high incidence of TSWV infection in *P. oleracea* in the field in Turkey, but reported that this species did not favour reproduction of *F. occidentalis*. However, this species and *S. oleraceus* may be less important as reservoirs of the virus than their abundance suggests if they only support low populations of larvae. Further work is needed to compare transmission from infected weed plants to economically important hosts like tomatoes.

Oxalis latifolia and *C. benghalensis* are perennial weed species identified as hosts of TSWV. *Oxalis latifolia* occurred frequently at Bungoma and Nakuru while *Commelina benghalensis* was common in all the production areas. Although these weeds supported relatively low thrips reproduction, their status as hosts of TSWV makes them play an important role as their perennial nature enables them to persist in the field for a long time and withstand harsh environmental conditions. Perennial weed species serves as persistent source of virus inoculum which is later passed onto annual weeds where it replicates before it is further spread to susceptible crops (Persley *et al.*, 2006; Wilson, 1998). This indicates that these weeds should not be ignored while developing management options for TSWV in tomato production.

Malva parviflora was an interesting weed species as it consistently supported high thrips reproduction, feeding damage and TSWV infection. Although no symptoms were observed on

the infected plants analysis of the asymptomatic plants through DAS-ELISA and RT-PCR indicated they were positive for TSWV. This finding is consistent with observations made by Bautista *et al.* (1995), who found a high incidence of asymptomatic TSWV infection and a strong attraction of *F. occidentalis* to *M. parviflora*. As a long-lived annual or perennial weed *M. parviflora* has the ability to remain longer in the field making it an important silent reservoir of TSWV.

In conclusion the study has revealed the occurrence of numerous weed species in major tomato production areas most of which can support TSWV and are hosts of *F. occidentalis*. Some of the weed species had special attributes which enhance their importance in TSWV epidemiology. This study highlights the importance of weed management as a component in the integrated management of TSWV and its vectors. However, there is need to further evaluate the reproduction potential and persistence of these weeds to better understand their contribution to TSWV epidemiology with the view of developing effective and cost-effective management options.

Chapter 6. Reaction of tomato cultivars grown in Kenya to *Tomato spotted wilt virus* (TSWV) infection.

6.1 Introduction

Tomato spotted wilt virus (TSWV) is an important tomato virus reported to cause severe yield losses in fresh market and processed tomato (*Solanum lycopersicum* L.) production around the world (Pappu *et al.*, 2009; Whitfield *et al.*, 2005). Its ability to infect a wide range of hosts and efficient transmission by *Frankliniella occidentalis* has led to the virus being rated among the most important plant viruses worldwide (German *et al.*, 1992; Parrella *et al.*, 2003; Scholthof *et al.*, 2011). *Tomato spotted wilt virus* induces a wide range of symptoms including stunting, reddish brown rings on leaves which coalesce into necrotic leaf spots, mosaic, mottling, purpling, epinasty, leaf and fruit deformity, chlorotic ringspot on fruits, and in severe cases, death of the plant (Aramburu *et al.*, 2000; German *et al.*, 1992). Apart from causing severe fruit yield losses, TSWV infection results in blemished fruit with necrotic or chlorotic ringspots that render the fruit unmarketable (Riley *et al.*, 2011a; Stevens *et al.*, 1995). *Tomato spotted wilt virus* is associated with severe yield losses in tomato production in many parts of the world with losses of up to 95% being recorded on susceptible cultivars (Pappu *et al.*, 2009; Riley *et al.*, 2011a).

Strategies for the management of TSWV through controlling thrips have been developed and utilised. The strategies such as reflective mulches (Riley & Pappu, 2004) and early season chemical application, cultural controls and weed control were found to be marginally effective

(Momol *et al.*, 2004; Riley & Pappu, 2004). This was attributed to the wide host range of the thrips and the virus, rapid development of resistance by vectors, effective transmission after short feeding periods, migration of thrips from outside the crop and the cryptic nature of the thrips (Gao *et al.*, 2012; Immaranju *et al.*, 1992; Jensen, 2000).

Use of plant host resistance has been regarded as the most sustainable management option for TSWV in tomato production (Gordillo *et al.*, 2008; Saidi & Warade, 2008; Soler *et al.*, 2003). Promising genes that confer resistance against TSWV have been identified from wild tomato, *Solanum peruvianum* and *S. chilense* and in pepper, *Capsicum chinense* (Canady *et al.*, 2001; Dianese *et al.*, 2011; Stevens *et al.*, 1994). The genes have been introgressed into different commercial cultivars leading to the development of resistant cultivars in tomato, and capsicum (Dianese *et al.*, 2010; Stevens *et al.*, 1994). Among the major genes identified that confer resistance, *Sw-5* has been rated as the most effective with broad resistance and has been incorporated in many tomato breeding lines and cultivars through conventional breeding (Saidi & Warade, 2008; Stevens *et al.*, 1994). The *Sw-5* gene triggers a hypersensitive response around the infection point limiting virus spread within the plant (Stevens *et al.*, 1994). The resistance arising from the *Sw-5* gene is highly stable and has been shown to have a broad spectrum resistance to other tospoviruses such as *Groundnut ringspot virus* (GRSV), *Tomato chlorotic spot virus* (TCSV) and *Chrysanthemum stem necrotic virus* (CSNV) (Boiteux *et al.*, 1993; É. C. Dianese *et al.*, 2010; Stevens *et al.*, 1994). Use of resistant cultivars has resulted in reduced losses from TSWV in commercial tomato production under high pressure of viruliferous thrips species (Dianese *et al.*, 2010; Giordano *et al.*, 2000; Stevens *et al.*, 1994).

Virus isolates able to overcome resistance conferred by the *Sw-5* gene have been identified in Hawaii, Australia, South Africa, Spain and Italy (Aramburu *et al.*, 2010; Ciuffo *et al.*, 2005; Latham & Jones, 1998; Thompson & van Zijl, 1996), leading to further research for additional reliable genes. The *Sw-7* gene has been identified to be effective against some of the resistance breaking races and is being introgressed into commercial lines (Stevens *et al.*, 2006). Although resistance breaking isolates have been identified, use of resistant cultivars is still the most important method for the management of TSWV in tomato production (Aramburu *et al.*, 2010; Stevens *et al.*, 1994).

TSWV has become established in tomato production in Kenya (Chapter 3). However, in this and other African countries where the disease has been introduced in recent years, information on the occurrence and utilisation of TSWV resistant cultivars is lacking. Therefore, the main aim of the current study was to establish the reaction to TSWV of commercial cultivars grown in Kenya. The cultivars available to growers were evaluated for the presence of the *Sw-5* gene using specific PCR primers and high resolution melt curve analysis.

6.2 Materials and Methods

6.2.1 Survey data on tomato cultivars grown in Kenya

Information on tomato cultivars grown in four major tomato production areas (Nakuru, Kirinyaga, Loitokitok and Bungoma) in Kenya was collected alongside data for TSWV and its vectors (Macharia, et al., 2015a). During the survey, farms were selected randomly and data on

tomato cultivars grown by farmers and their ability to resist TSWV according to farmers' observations were recorded.

6.2.2 Tomato cultivars

Seeds of all tomato cultivars available commercially in Kenya were purchased. In cases where the same variety was sold by different companies, only seeds from one company were used. A total of 42 tomato varieties (Table 6.1) were collected from 8 seed companies which consisted of six greenhouse varieties, three dual-purpose greenhouse and open field varieties and 33 open field varieties. Greenhouse varieties were all indeterminate, while field varieties included both determinate and indeterminate forms. Twenty of the cultivars were F1 hybrids. Seventy six percent of the tomato cultivars were reported to contain resistance to fungal, bacterial, viral and/ or nematode diseases (Table 6.1). The cultivars had fruit shapes that ranged from round, irregular, or globose to oval or roma type. Cultivars Swanson (resistant to ToMV, TSWV, Fusarium wilt, Verticillium wilt and root knot nematode) and Klass (ToMV, TSWV, Fusarium wilt, verticillium wilt, Fusarium root rot and root knot nematode) were used as positive controls while detecting the Sw-5 gene in the Kenyan samples.

6.2.3 TSWV isolate

The TSWV isolate N55 used in the greenhouse experiments was isolated from an infected tomato plant collected from the Nakuru area (Chapter 3). Infected tomato leaves were ground in 0.1 M phosphate buffer pH 7.4 with 1% sodium sulphite and the resulting suspension was mechanically inoculated on *Datura stramonium* and *Nicotiana benthamiana* leaves dusted with carborundum

powder. The plants were rinsed with tap water to remove excess sap and carborundum powder. The indicator plants were maintained in an insect-proof greenhouse and observed for symptom expression. Infection from the isolate resulted in characteristic TSWV symptoms on *D. stramonium* (stunting, mosaic, chlorosis, mottling, leaf distortion, severe necrosis and wilting) and *N. benthamiana* (stunting, mosaic, leaf distortion and wilting). Infection was confirmed to be due to TSWV with DAS-ELISA and RT-PCR, which were performed as described in Chapter 3. The partial nucleocapsid gene was sequenced (Chapter 3) and the sequence had a high nucleotide sequence similarity ranging from 97 to 99% with other TSWV sequences in GenBank. The virus was multiplied and maintained on *D. stramonium* and susceptible tomato cultivars in an insect-proof greenhouse while backup isolates were stored at −80^{o}C.

Table 6.1. Tomato cultivars used in the evaluation for resistance to *Tomato spotted wilt virus* and their disease resistance status

Variety	Seed company[1]	Disease resistance[2]	Growth type	Open field or greenhouse
Claudia	Amiran	bfg	Indeterminate	Greenhouse /Open field
Shanty	Amiran	adfgm	Determinate	Open field
Nemo Netta	Amiran	defgm	Indeterminate	Greenhouse
Cal J	East African		Determinate	Open field
Nuru F1	East African		Indeterminate	Greenhouse
Heatmaster F1	Monsanto	dfghjm	Determinate	Open field
Veloz F1	Monsanto	abdfgm	Determinate	Open field
Dominator (Assila F1)	Monsanto	bdfgm	Determinate	Open field
Loreto (Anna F1)	Monsanto	fgjm	Indeterminate	Greenhouse /Open field
Eden F1	Monsanto	dfghjim	Determinate	Open field
AB2	Monsanto	fgi	Determinate	Open field
Nema 1400	Monsanto	fgm	Determinate	Open field
Picus F1	Monsanto	abdfghjm	Determinate	Open field
Alex 63 F1	Monsanto		Determinate	Open field
DRG 8564	Monsanto	abdfgm	Determinate	Open field
Sandokan F1	Royal	**a**fglm	Determinate	Open field
Rambo F1	Royal	fgkm	Determinate	Open field
Oxly Premium F1	Royal	fgj	Determinate	Open field
Mavuno F1	Royal	dfkm	Indeterminate	Open field
Chonto F1	Royal	bcdfghm	Indeterminate	Greenhouse
Oxly	Royal	d	Determinate	Open field
Bravo F1	Royal	bfghm	Indeterminate	Greenhouse
Xewel F1	Savannah	bef	Determinate	Open field
Jaguar F1	Savannah	bf	Determinate	Open field
Ganila F1	Savannah	fe	Determinate	Open field
Lucy F1	Savannah	f	Determinate	Open field
Kiara F1	Savannah	bf	Determinate	Open field
Tima	Savannah	g	Determinate	Open field
Lindo F1	Savannah	efk	Determinate	Open field
Moneymaker	Simlaw		Indeterminate	Open field
Nouvelle	Simlaw		Determinate	Open field
Heinz 1350 VF	Simlaw		Determinate	Open field
Marglobe	Simlaw		Determinate	Open field
Fortune Maker F1	Simlaw		Indeterminate	Greenhouse
M82	Simlaw	fg	Determinate	Open field
Prostar F1	Simlaw	bk	Indeterminate	Greenhouse /Open field
Rio Grande	Simlaw	fg	Determinate	Open field
Gold Nugget	Starke Ayres	defgm	Determinate	Open field
Little Wonder	Starke Ayres		Determinate	Open field
Roma VFN	Starke Ayres	fgm	Determinate	Open field
Tylka F1	Sygenta	bdfgim	Indeterminate	Greenhouse
Kilele F1	Sygenta	bdfgm	Determinate	Open field

[1]Seed companies from where seed were purchased

[2]Disease resistance: TSWV[a], TYLCV[b], CMV[c], ToMV[d], TMV[e], Fusarium wilt[f], Verticillium wilt[g], grey leaf spot[h], Fusarium crown rot[i], Alternaria stem canker[j], bacterial wilt[k], bacterial speck[l], nematode[m]

6.2.4 Screening tomato cultivars for resistance to TSWV under greenhouse conditions

Greenhouse experiments were carried out at the Kenya Plant Health Inspectorate Services (KEPHIS), Plant Quarantine and Biosecurity Station in Muguga (1.213° S, 36.631° E, elevation 2082 m asl) located 30 km west of Nairobi. The cultivars were first sown in propagation trays with potting medium and maintained in an insect-proof greenhouse. Three to four weeks after sowing when seedlings had attained at least 2 pairs of true leaves, they were transferred to 4 litre pots containing steam sterilised medium (mix of forest soil, manure, gravel and sand). Ten plants were prepared for each cultivar and the experiment was arranged in a completely randomised design. The plants were mechanically inoculated with TSWV isolates after establishment when they had attainied at least 3 pairs of true leaves. Infected *Datura stramonium* leaves were ground in 0.1 M phosphate buffer pH 8.0 with 1% sodium sulphite and the sap used to inoculate plants that were dusted with carborundum powder. Negative controls were dusted with carborundum powder and inoculated with buffer only. The plants were re-inoculated after 7 days to maximise infection and avoid escapes. The plants were maintained in an insect proof greenhouse at 25±2 °C. The experiment was carried out twice, with all the 42 varieties in the first trial and 39 varieties in the second. This was because of poor germination experienced with Ganilla, Nemo Netta and Gold Nugget in the second trial.

The plants were evaluated for TSWV infection 30 days after inoculation. The plants were visually examined for TSWV symptom expression and disease severity was rated on a scale of 1–5 according to Canady *et al.* (2001) with modifications, where 1 = no symptoms, 2 = purpling, chlorosis and limited leaf distortion, 3 = purpling, chlorosis, leaf necrosis and leaf distortion with some stunting, 4 = purpling, chlorosis, leaf necrosis, leaf distortion and plant stunting, 5 =

purpling, chlorosis, leaf necrosis, leaf distortion and extreme stunting. Incidence was scored as the total number of infected plants out of the total number of plants inoculated per cultivar. TSWV infection was confirmed using the DAS-ELISA kit (Agdia, Elkhart, IN, USA) which was performed according to the manufacturer's specifications. The plants were rated as positive if the optical density (OD) from the ELISA reader was at least three times the average reading of the healthy control. The ELISA reading was used to indicate the relative virus titre in different tomato cultivars. The data on severity and optical density from ELISA were subjected to analysis of variance (ANOVA) and means were separated using least significant difference (LSD) in Genstat 17th edition (VSN International, 2014)

6.2.5 Reaction of different tomato varieties to TSWV and its vector under field conditions

Field experiments were carried out in Nakuru and Kirinyaga areas which represented two agro-ecological zones where tomatoes are produced. Thirty selected cultivars (Table 6.4) were evaluated for resistance to TSWV in small plots measuring 4 m x 3 m with spacing of 45 cm by 90 cm with natural TSWV infection. Each treatment was replicated 3 times in a complete randomized block design. The cultivars were first sown in 128 cell Styrofoam propagation trays containing potting mixture and maintained in an insect-proof greenhouse. The seedlings were transplanted in the field after attaining at least 10–15 cm in height. The field plots were maintained free of weeds and fungal diseases were controlled using fungicides. The plants were observed for symptom expression, and data on incidence and severity of TSWV was determined as above. *Tomato spotted wilt virus* infection was tested using DAS-ELISA. Thrips were monitored in the field trial using blue sticky traps. The population of thrips on each cultivar was established 60 days after planting by tapping plants over a white surface and collecting thrips

with a soft brush into vials containing 80% ethanol. The species that were collected from the trial were identified using morphological keys. Only major thrips species reported as vectors of TSWV were identified to species level.

6.2.6 Evaluation of the *Sw-5* gene in tomato cultivars

The presence of the *Sw-5* gene in tomato cultivars grown in Kenya was tested using PCR based methods developed by É. C. Dianese *et al.* (2010) and Shi and Vierling (2011). DNA was extracted from the tomato plants using ZR Plant/Seed DNA MiniPrep (Zymo Research Corporation, CA, USA) according to the manufacturer's specification. The DNA was eluted using 50 µl of DNA elution buffer and the resulting DNA was spotted on FTA cards and allowed to dry. DNA was eluted from the FTA cards using TE buffer, where 1 cm^2 discs were soaked in 200 µl of TE buffer and incubated for one hour at 4°C. The DNA was squeezed out from the discs after incubation. The resulting DNA was used in PCR amplification using *Sw-5* specific primers. The polymorphic primers used were: *Sw*-5-2F (5'-AAT TAG GTT CTT GAA GCC CAT CT-3') and *Sw-5*-2R (5'-TTC CGC ATC AGC CAA TAG TGT-3') that amplify the fragments with different sizes of amplicons representing the resistant (574 bp) and susceptible (464 bp) alleles (É. C. Dianese *et al.*, 2010). Primers *Sw-5*-f2 (5'-CGG AAC CTG TAA CTT GAC TG-3') and *Sw-5*-r2 (5'-GAG CTC TCA TCC ATT TTC CG-3') amplify the resistant allele only (Shi & Vierling, 2011).

Polymerase chain reaction amplification was carried out using 2 µl of the DNA in a 20 µl reaction mix containing 2 µl of 10× PCR buffer (Bioline, Sydney, Australia), 1.5 mM $MgCl_2$, 0.2 mM dNTP, 0.2 µM of each primer, and 0.5 U of Biotaq DNA polymerase (Bioline). The

amplification was performed in a DNA Engine Peltier Thermal Cycler (BioRad, CA, USA) using the programs: a) for *Sw-5*-F and *Sw-5*-R; denaturation at 94^{o}C for 2 min, followed by 29 cycles of 94°C for 30 s, 50°C for 1 min and 72°C for 30 s and final extension at 72°C for 5 min; and b) for *Sw-5*-f2 and *Sw-5*-r2 at 95°C for 2 min, followed by 36 cycles of 94°C for 15 s, 56°C for 15 s and 72°C for 45 s and a final extension at 72°C for 5 min. PCR products were visualised in a 1.5% agarose gel.

6.2.7 Assessment of single nucleotide polymorphisms (SNPs) through high resolution melt curve analysis (HRM)

High resolution melt curve analysis was used for detection of single nucleotide polymorphisms (SNPs) using specific primers published by Shi and Vierling (2011); *Sw-5*-SNP1 ATF (5'-ACCCACTTCTTCAAGTCGAG-3') and *Sw-5*-SNP1 ATR (5'-GGT ATG GGA TGA CTT AAG GC-3'); *Sw-5*-SNP2 AGF (5'-TTT CTC CTC AAC ATG TCG GC-3') and *Sw-5*-SNP2 AGR (5'-GAG AGA GTT ATT ACA AGA T-3'). An additional primer pair (*Sw-5*-HRM1) was also designed and tested in the current study. The sequences AY007366, FJ686041, FJ686043 and FJ686044 representing TSWV resistant protein and FJ686039, FJ686040 and FJ686042 representing the susceptible protein from GenBank were aligned using ClustalW to identify SNPs (Fig. 6.1). A pair of primers *Sw-5*-HRM1 F (5'-CAT CAA CCG TAG CTC CAT TGT C-3') (305-325) and *Sw-5*-HRM1 R (5'-CGA GCA TGG TGC ATC ATT TC-3') (411-433) with an expected size of 128 bp was designed using Primer3 targeting 2 SNPs (T/C and A/G) from the aligned sequences. The primers were analysed using NetPrimer to detect possible secondary structures, such as primer dimer, hairpin, palindrome, and repeats (http://www.Premierbiosoft.com/ netprimer/netprimer.html, Premier Biosoft International, Palo

Alto, CA), and ΔG of the secondary structure of the amplicon was analysed using the DINAMeltServer (http://mfold.rna.albany.edu/?q=dinamelt) (Markham & Zuker, 2005).

The quantitative real-time PCR amplifications were carried out using 2 µl of the DNA (20 ng/µl) in a 20 µl reaction volume which comprised 2 µl of 10× PCR buffer (Bioline), 2.5 mM $MgCl_2$, 2.0 mM dNTP, 0.3 µM of each primer, 1.5 µM of Syto 9, and 0.05 U of Biotaq DNA polymerase (Bioline). The PCR was performed in a Rotor-Gene 6000 real-time PCR Thermocycler (Corbett Research, Sydney, Australia) using a touchdown program as follows: first denaturation at 94^{o}C for 2 min, followed by 14 cycles of denaturation at 94°C for 5 s, annealing at 62°C for 20s with 0.5°C decrease each cycle, and extension at 72°C for 30 s, then 20 cycles of denaturation at 94°C for 5 s, annealing at 55°C for 20 s and final extension of extension at 72°C for 2 min. High resolution melting curve analysis (HRM) was done after the PCR amplification by increasing the temperature by 0.1°C from 60–87 °C and data collected was analysed using the Rotor-Gene 6500 series software, Corbett Research (Wu *et al.*, 2008). The resulting PCR product was visualised in a 1.5% agarose gel. The HRM curve which indicates differences in nucleotide sequence between the different genotypes was visually scored for each individual cultivar. Differences between the cultivars were identified by examining the normalised, difference, and derivative melt plots.

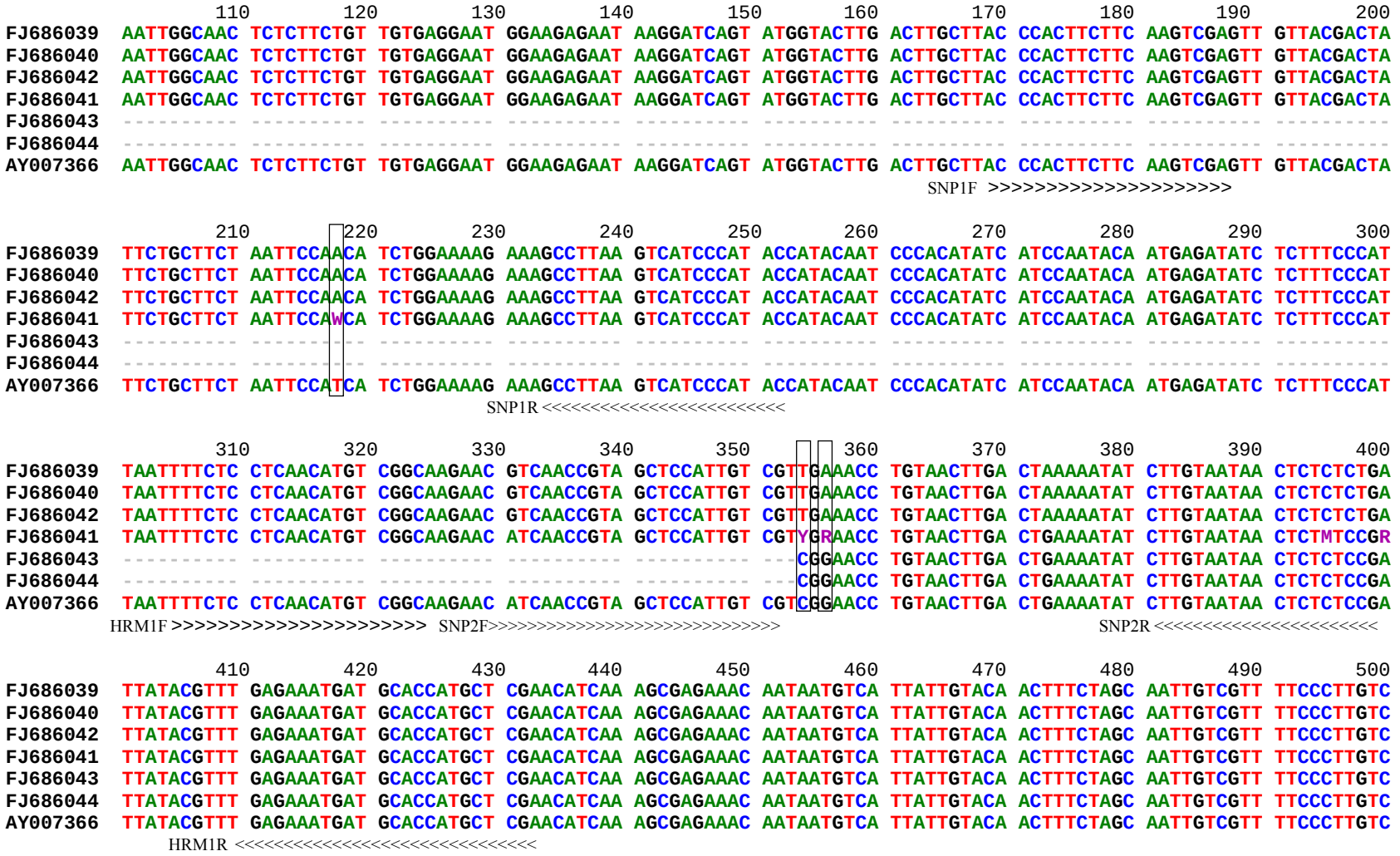

Figure 6.1 Alignment of seven tomato sequences (3 susceptible and 4 resistant) indicating three SNPs, A/T, T/C and G/A and position of the three SNPs primer sets used in HRM

6.3 Results

6.3.1 Tomato cultivars grown in Kenya

At least thirteen tomato cultivars were grown in different production areas (Table 6.2). Rio Grande, Kilele and Oxly were the most cultivated varieties and only 4% of farms did not have one of these as their major variety. Rio Grande was the main variety on more than 63% of farms and was the only variety that was being cultivated in all four production areas. Rio Grande was particularly the most preferred variety in Nakuru (90%) and Loitokitok (93%) (Table 2). Kilele was the second most widely grown tomato cultivar and most preferred in Kirinyaga, while Oxly was the main variety in Bungoma. None of other varieties being cultivated were reported to be resistant or tolerant to TSWV except Sandokan which was being grown in only one farm as a trial. Farmers were found to either purchase new seeds or used their own seeds saved from the previous crop (Table 6.3). Famers in Bungoma and Loitokitok mainly purchased new seeds each season while in Nakuru and Kirinyaga larger numbers of farmers used their own seeds. Over 90% of the farmers in Kirinyaga used their own seeds.

6.3.2 Screening tomato cultivars for resistance to TSWV under greenhouse conditions

In the first greenhouse screening trial, 28 out of 42 tomato cultivars showed positive ELISA reactions in at least one plant and were considered susceptible to TSWV (Table 6.4). This comprised 44% of the greenhouse varieties and 70% of the open field varieties (Table 6.4). Infected tomato cultivars produced characteristic TSWV symptoms which included stunting, purpling, chlorosis, necrosis, mosaic, leaf distortion, leaf curl and concentric rings on leaves (Table 6.4). Stunting, chlorosis and purpling were the most commonly observed symptoms on

most of the susceptible varieties (Fig. 6.4). Severe necrosis was observed on NEMA 1400, Xewel, Nouvelle and Lindo while necrotic rings on leaves were observed on Nouvelle, Lindo, Rambo, Eden and Rio Grande.

Table 6.2 Tomato cultivars grown in the four major tomato production areas. Data are the number of farms which grew each cultivar.

Variety	**Frequency**	**Area**			
		Nakuru	**Kirinyaga**	**Loitokotok**	**Bungoma**
Assila F1	1	–	–	1	–
Bravo F1	1	1	–	–	–
Cal J	3	2	–	–	1
Eden Fi	1	–	1	–	–
Faulu F1	1	–	1	–	–
Kilele	19	–	19	–	–
Onyx	3	–	–	–	3
Oxly	13	2	1	–	10
Oxly Premium	1	–	1	–	–
Rio Grande	80	43	12	13	12
Rambo	1	–	1	–	–
Sandokan	1	–	1	–	–
Valoria	1	–	1	–	–

Table 6.3 Source of tomato seeds used in the production areas

Source	**Area**			
	Nakuru	**Kirinyaga**	**Loitokotok**	**Bungoma**
Purchased new seeds	25	3	9	14
Saved own seeds	18	28	5	5
Purchased new seeds and own seeds	2	-	-	-

There was a significant difference ($P < 0.001$) in disease severity among the cultivars. The highest severity was recorded on Prostar and among those that did not succumb to infection, Jaguar had the lowest severity. High disease incidence was recorded on Nema 1400, Moneymaker, Eden and Xewel with more than six plants infected (Table 6.4). Similarly, virus titre based on optical density from DAS-ELISA was significantly different ($P < 0.001$) among the cultivars. High levels were recorded in Eden, Nema 1400, Lindo and Nuru. Rio Grande which accounted for over 59% of the tomato production in Kenya was susceptible to TSWV. There was a significant positive correlation ($r = 0.691$; $P < 0.01$) between severity and virus titre based on ELISA readings among the different cultivars with those showing high severity also having higher incidence and higher virus titres. However, 14 cultivars produced no symptoms and tested negative with DAS-ELISA and were therefore were rated as resistant or tolerant to TSWV (Table 6.4).

A repeat experiment was done with 39 cultivars (Table 6.5). The varieties Moneymaker, Nema 1400, Eden and Xewel consistently showed higher disease severity, incidence and virus titre equivalence in both experiments. However, Alex 63 F1, Dominator, Mavuno, Oxly and Sandokan consistently showed low incidence and virus titre. Several varieties including Shanty, Veloz, DRD 8564, Chonto, Little Wonder, Heatmaster, Heinz 1350 VF, Loreto and Lucy consistently remained negative against TSWV (Table 6.5). Although Kilele was positive in the previous experiment, it tested negative in the repeat experiment. The varieties AB2, Picus, Jaguar, Kiara F1, Tima and Bravo exhibited no symptoms.

Figure 6.2 Tomato cultivars infected with *Tomato spotted wilt virus*: a) variety Eden showing stunting and necrotic spots, b) Lindo showing stunting and chlorosis, c) Moneymaker showing stunting and purpling and d) Xewel showing stunting and concentric rings on leaves. Inserted images represent infected part of the tomato plant.

Table 6.4 Reaction of tomato cultivars to *Tomato spotted wilt virus* infection under greenhouse conditions in the first trial

Variety	Symptoms[a]	Severity	Incidence[b]	ELISA OD[c]
Nema 1400	ST, P, NS, SN	3.1	7/9	2.49
Moneymaker	ST, P, NS	3.2	7/9	1.69
Eden F1	ST, P, C, NS	3.8	6/9	2.58
Xewel F1	ST, C, NS	3.1	6/9	1.20
Lindo F1	ST, CN, NR, C	3.4	3/9	2.88
Rio Grande	ST, P	2.8	3/9	2.18
Gold Nugget	ST, P, C, LD, NS	4.0	3/9	1.82
Claudia	ST, P, LD, CR, M	3.3	3/9	1.47
Nuru F1	ST, P, C	2.5	2/9	2.37
Prostar F1	ST, P, C,W, NS	4.5	2/9	1.91
M82	P, LD, LC, SN, M	4.0	2/9	1.73
Jaguar F1	NS, P	1.5	2/9	1.20
Oxly	SN, P, M, C	2.9	2/9	1.15
Mavuno F1	ST, P, C, LD	4.0	2/9	0.91
Dominator (Assila F1)	ST, P	3.3	2/9	0.81
Tylka F1	ST, P, NS,	4.2	1/9	1.87
AB 2	P, C	2.3	1/9	1.67
Fortune Maker F1	ST, P, NS	4.0	1/9	1.42
Alex 63 F1	C, P, SN, LD	3.5	1/9	1.40
Marglobe	ST, C, NS	3.5	1/9	1.39
Roma VFN	P, LC, C, LD, SN	3.0	1/9	1.38
Kilele F1	ST, C, P, NS	4.0	1/9	1.37
Kiara F1	P, C, ST, NS, LD	4.0	1/9	1.24
Cal J	ST, P, C, NS	3.0	1/9	0.96
Rambo F1	ST, P, NS	3.0	1/9	0.92
Nouvel	ST, P, C, M, LD, NS	3.5	1/9	0.83
Sandokan F1	ST, P, LD, NS	3.0	1/9	0.65
Oxly Premium F1	ST, P, LD, C	3.5	1/9	0.30
Bravo F1		1.3	0/9	0.10
Shanty		1.0	0/9	0.10
Tima	P	1.9	0/9	0.10
Little Wonder		2.0	0/9	0.10
Ganila F1	P	1.8	0/9	0.10
DRD 8564		1.3	0/9	0.10
Heatmaster F1		1.4	0/9	0.10
Nemo Netta		1.0	0/9	0.10
Picus F1		1.3	0/9	0.10
Veloz F1	P	1.0	0/9	0.10
Lucy F1	P, ST	1.9	0/9	0.10
Heinz 1350 VF	C, NS	1.0	0/9	0.09
Loreto (Anna F1)	S	1.8	0/9	0.09
Chonto F1		1.0	0/9	0.09
LSD ($P < 0.001$)		0.78		0.201

[a] Symptoms observed on tomato plants CR- Concentric ringspot, NS-Necrotic spot, M-Mosaic, LD-leaf deformation, ST-stunting, W-wilting, P- Purpling, C-Chlorosis, SN-Severe necrosis

[b] Plant was rated positive if it had a plant that tested positive with ELISA

[c] Represent the average of the positive samples

Table 6.5 Reaction of tomato cultivars to *Tomato spotted wilt virus* infection under greenhouse conditions in the second trial

Variety[a]	Symptoms[b]	Severity	Incidence[c]	ELISA OD
Moneymaker	ST, P, C, NS ,M, LD	3.9	9/9	1.70
Xewel F1	ST, P,C, NS, M, LD, NR, CR	3.4	8/9	2.61
Eden F1	ST, P,C,NS,M,LD	3.6	7/9	2.59
Nema 1400	ST, P, C, NS, M, LD	3.1	7/9	2.49
Lindo F1	ST, C, NS, CR, LC, NR	4.0	4/9	2.97
Cal J	ST, P, C, NS, M, LD	3.3	4/9	1.27
Prostar F1	ST, P, C, M, LD	3.3	4/9	-
M82	ST, P, C ,NS, M	3.0	4/9	-
Oxly Premium F1	ST, P, LD	4.0	3/9	2.77
Rambo F1	ST, P, C, NS	3.7	3/9	3.27
Claudia	ST, P, C, NS, M, LD	3.3	3/9	1.39
Roma VFN	ST, C, M	3.3	3/9	-
Nouvel	ST, C, NS, CR	3.0	3/9	3.35
Rio Grande	ST, P, C, NS, CR, LC	3.0	3/9	2.18
Fortune Maker F1	ST, P, LD	3.0	3/9	1.70
Marglobe	ST, C, M	3.0	3/9	1.49
Mavuno F1	ST, P, C, NS, LD	4.0	2/9	1.32
Nuru F1	ST, C, NS, LD	3.0	2/9	2.17
Oxly	ST, P, M	3.0	2/9	1.38
Sandokan F1	ST, P, C, NS, M, LD	4.0	1/9	1.89
Dominator (Assila F1)		3.0	1/9	1.30
Alex 63 F1		1.0	1/9	1.28
Tylka F1	ST, P, C, M, LD	1.6	0/9	-
Shanty		1.1	0/9	0.09
Kilele F1		1.0	0/9	0.11
Chonto F1		1.0	0/9	0.10
Little Wonder		1.0	0/9	0.10
Heatmaster F1		1.0	0/9	0.10
Veloz F1		1.0	0/9	0.10
DRD 8564		1.0	0/9	0.10
Heinz 1350 VF		1.0	0/9	0.09
Loreto (Anna F1)		1.0	0/9	0.09
Lucy F1		1.0	0/9	0.09
AB 2		1.0	0/9	nt
Picus		1.0	0/9	nt
Jaguar		1.0	0/9	nt
Kiara F1		1.0	0/9	nt
Tima		1.0	0/9	nt
Bravo		1.0	0/9	nt
LSD ($P < 0.001$)		0.44		0.71

- Cultivars not tested with ELISA

[a] Varieties Ganilla, Nemo Netta and Gold nugget had poor germination hence were not included in the trial.

[b] Symptoms observed on tomato plants CR- Concentric ringspot, CS- chlorotic spot, NR-Necrotic ringspot, NS-Necrotic spot, M-Mosaic, Mo-Mottle, LD-leaf deformation, ST-stunting, W-wilting, P- Purpling, C-Chlorosis, D-Leaf death, SN-Severe necrosis

[c] Cultivars were rated as positive if they had plants that tested positive with ELISA

nt- not tested

6.3.3 Reaction of different tomato varieties to TSWV and its vector under field conditions

The plants were evaluated for TSWV symptoms at 30 and 60 days after planting (DAP) and no symptoms characteristic of TSWV were observed on any of the cultivars under evaluation in both trial sites. However, the field trial could not be extended beyond 60 days due to restrictions on the time allowed for field work in Kenya.

Low thrips populations were observed in both trial sites with higher thrips populations observed in the Kirinyaga area. The population differed significantly among tomato cultivars ($P < 0.05$) in the Kirinyaga and Nakuru field trials. Cultivar Tima had the highest thrips population in Kirinyaga (17.7) and Nakuru (11.0), while Kilele F1 had the second highest thrips population within the two trials (Table 6.6). Little Wonder had the lowest thrips population of 2.6 and 2.3, in Kirinyaga and Nakuru trial, respectively (Table 6.6). There were no clear differences in thrips population between TSWV-resistant and susceptible cultivars in either field trial. Thrips species collected were comprised of males and females of *Frankliniella occidentalis, Thrips tabaci, Ceratothripoides brunneus, F. schultzei* and *Haplothrips* species.

Table 6.6 Means of thrips population from the two field trials in Nakuru and Kirinyaga*

Variety	**Kirinyaga[a]**	**Nakuru[b]**
AB2	7.8	3.9
Alex 63 F1	6.8	3.8
Cal J	5.1	1.1
Claudia	6.9	2.7
Dominator (Assila F1)	6.3	3.7
DRD 8564	5.2	3.6
Eden F1	3.0	3.1
Heatmaster F1	8.8	2.0
Heinz	5.4	3.5
Jaguar F1	6.3	6.3
Kiara F1	4.9	2.3
Kilele F1	11.0	7.4
Little Wonder	2.6	2.3
Loreto (Anna F1)	4.6	3.3
Lucy F1	4.6	2.5
M82	4.3	3.5
Marglobe	4.1	2.7
Mavuno F1	5.9	3.2
Moneymaker	4.2	1.5
Nema 1400	5.8	3.3
Oxly	5.7	2.1
Oxly Premium F1	3.7	2.5
Rambo F1	6.4	1.1
Rio Grande	7. 7	2.5
Roma VFN	6.0	2.4
Sandokan F1	5.5	2.9
Shanty	6.9	2.2
Tima	17.7	8.5
Veloz F1	5.6	3.3
Xewel F1	8.5	1.9
LSD ($P < 0.001$)	1.68	1.84

[a,b] Thrips population is from an average of 15 plants representing five plants in each of the three blocks

* The trials were set up to screen tomato cultivars for resistance to TSWV

6.3.4 Evaluation of the *Sw-5* gene in tomato cultivars

Tomato cultivars were evaluated for the *Sw-5* gene that confers resistance to TSWV. Six tomato cultivars: Sandokan, Shanty, Picus, AB2, Veloz and DRG 8564 were shown to carry the *Sw-5* gene using both the polymorphic primers and the primers for the resistant allele. All 6 cultivars gave two bands of the expected sizes for the resistant and susceptible alleles using the polymorphic primers. Most of the cultivars containing the *Sw-5* gene tested negative to TSWV infection under greenhouse evaluations except Sandokan and AB2 which showed low infection to TSWV. Tomato cultivars Tima and Oxly Premium two produced bands with the polymorphic primers with amplicon sizes (464 and 510 bp) that were different from the resistant alles (574 bp). These cultivars tested negative with the second set of primers (*Sw-5* f2 and r2) hence were rated as heterozygous susceptible. The rest of the cultivars produced a single band of 464 bp with the polymorphic primers but did not produce any band with the second set of primers.

6.3.5 Assessment of *Sw-5* SNPs through high resolution melt curve analysis

The high resolution melting (HRM) curve analysis protocol for identification of SNPs was undertaken for four tomato cultivars using the three sets of SNP primers. The first primer pair (*Sw-5*-SNP1 AT) targeting the A/T SNP gave amplification and the HRM analysis produced two sets of that curves showed a clear but small difference between the susceptible and the resistant cultivars (Fig. 6.3a). However, no amplification was observed with the second primer pair (*Sw-5*-SNP2 AG). On the other hand, the third primer pair (*Sw-5*-HRM1 F & R) designed in this study showed a clear segregation between the resistant and susceptible cultivars. Varieties Klass and Swanson produced a curve with two melting phases which indicated heterozygosity, while Moneymaker and Grosse Lisse varieties only showed a single melting phase which suggests

homozygosity of the genotype (Figure 6.3b). The observation was supported by derivative melting curve analysis where a single peak was observed for the susceptible varieties and double peaks for the resistant varieties (Fig. 6.3c & d).

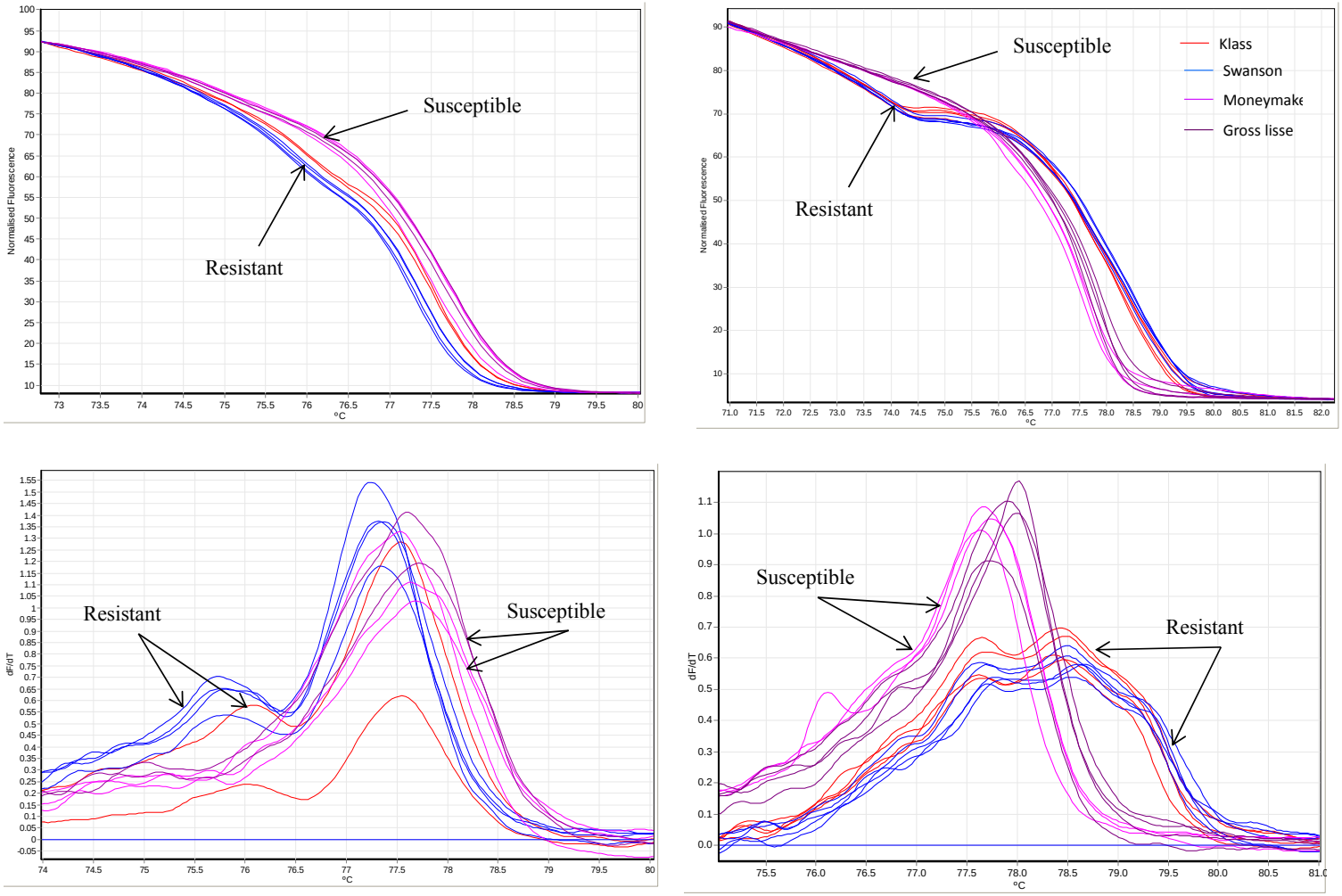

Figure 6.3 HRM profiles for 4 tomato cultivars analysed using primers *Sw*-5-SNP1 AT (a and c) and *Sw*-5-HRM1 (b and d). Parts a and b show the HRM melt curves; c and d show the derivative melting curves

6.4 Discussion

Tomato cultivars showed varied reactions to TSWV infection with at least two thirds of the cultivars available in Kenya rated as susceptible. This was comprised of half of the greenhouse cultivars and two-thirds of the open field cultivars. The susceptible cultivars produced characteristic TSWV symptoms while the resistant cultivars had either no symptoms observed or had a hypersensitive reaction with no further infection as previously reported in susceptible and resistant cultivars, respectively (German *et al.*, 1992; Pappu *et al.*, 2009; Riley *et al.*, 2011a). Moneymaker, Rio Grande, Cal J, Roma and Marglobe were previously reported to be susceptible to TSWV (Ramkat *et al.*, 2006), which was consistent with observations in the current study. Although resistant cultivars were identified among the cultivars available in Kenya, none of the cultivars being grown on a regular basis was resistant to TSWV. Rio Grande which is one of the most preferred tomato cultivars in the major production areas (Chapter 3), was consistently rated as susceptible to the virus. Therefore, there is a need to create awareness of the benefits of using resistant cultivars in the management of TSWV and to evaluate the preference of farmers and markets for the resistant cultivars.

Tomato cultivars Moneymaker, Nema 1400, Eden and Xewel which are consistently highly susceptible to TSWV should be avoided in areas where TSWV has been reported to occur. However, cultivars that showed low infection rate could be suitable for cultivation but there is a need to further evaluate them under field condition to establish their suitability. The varieties should also be subjected to screening using thrips inoculation to confirm the low level of infection. Field evaluation of cultivars for resistance to TSWV is important, as the field environment provides an opportunity for challenging the cultivar under different inoculum levels and weather conditions (Sivparsad & Gubba, 2011). Although results from

the field trials can be variable, it has been reported to be important in confirmation of virus resistance identified through confined greenhouse trials (Saidi & Warade, 2008). Sporadic occurrence of the disease which was observed in the production areas during the current study have been reported in previous studies (Riley *et al.*, 2011a) hence needs to be considered during field screening. Field evaluation of tomato cultivars against TSWV and its vectors is important to supplement the greenhouse data.

The cultivars were evaluated for the presence of the *Sw-5* gene that confers resistance to TSWV. Only 14 % of the evaluated cultivars contained the *Sw-5* gene. These cultivars did not show any systemic infection, but produced hypersensitive response as was observed in earlier studies (Rosello *et al.*, 1998). Varieties Sandokan and AB2 with *Sw-5* gene had a low level of infection to TSWV which could be attributed to mixing of seeds with susceptible varieties during processing or possibly some contamination of pollen during breeding. Similar observations were made where two hybrids containing the *Sw-7* gene had plants that tested positive to TSWV during field screening in Georgia, USA (Riley *et al.*, 2011a). Cultivars Shanty and Picus were shown to be consistently resistant to TSWV under field evaluation (Riley *et al.*, 2011a) where resistant cultivars consistently produced marketable fruit over a period of 5 years. Cultivars containing the *Sw-5* gene have been utilised in successful management of TSWV (Boiteux *et al.*, 1993; Cho *et al.*, 1996; Cho *et al.*, 1989; Riley *et al.*, 2011a; Rosello *et al.*, 1996). However, there is a need to use the cultivars judiciously to avert the possibility of TSWV genome reassortment due to over use in areas with high disease pressure (Gordillo *et al.*, 2008).

The PCR evaluation of the cultivars resulted in their classification into resistant or susceptible based on the *Sw-5* resistance gene. The sequence-derived PCR based markers

targets specific gene/alleles (Dianese *et al.*, 2010; Garland *et al.*, 2005; Shi & Vierling, 2011). The polymorphic PCR primers produced three patterns from the cultivar analysed. Resistant cultivars produced double amplicons of 464 and 575 bp in size. This was different from what was earlier reported where resistant varieties produced a single band (575 bp), (Dianese *et al.*, 2010), because all resistant varieties grown in Kenya were F1 hybrids and so were heterozygous for this allele. The results were verified using a different primer pair developed by Shi and Vierling (2011) where only resistant cultivars produced a single amplicon. The observation was compared with two resistant tomato cultivars Klass and Swanson that were known to contain the resistant *Sw-5* gene or alleles. Consequently, the susceptible cultivars produced two groups comprising of Tima and Oxly premium which had double amplicons between 464 and 510 bp and other susceptible cultivars which had an amplicon size of 464 bp. The analysis of *Sw-5* using both sets of primers produced similar results on cultivars with *Sw-5* gene. However, the polymorphic primers provide more information about the cultivars while the second set of primer provided a clear difference between the negative and the resistance cultivars. The use of specific PCR primers was found to provide an easy method for evaluation of resistance in different tomato cultivars (Dianese *et al.*, 2010; Garland *et al.*, 2005; Shi & Vierling, 2011). Furthermore, analysis of sequences derived from the PCR primers indicated that the primer pairs were specific for *Sw-5* gene locus and their differences were due to insertion/deletion (Dianese *et al.*, 2010; Shi & Vierling, 2011).

The presence of the *Sw-5* gene was further evaluated using high resolution melting curve (HRM) analysis with four tomato cultivars. HRM analysis produced two distinct curves discriminating the resistant and susceptible cultivars using the first (*Sw-5*-SNP1) and third SNPs primer (*Sw-5*-HRM1), respectively. This was due to fluorescence change during DNA

melting that resulted from differences in nucleotide sequence in between the different genotypes. HRM has been identified as a powerful method for detection of genetic variation including SNPs, insertion or deletion (Distefano *et al.*, 2013). It has been shown to be an efficient, accurate and inexpensive method for detection of polymorphism especially SNPs (Distefano *et al.*, 2012; Wu *et al.*, 2008). High resolution melt curve analysis has been utilised to study genetic variability in apple, barley, grapes, almond, citrus, pepper and sweet cherry (Distefano *et al.*, 2012; Distefano *et al.*, 2013; Wu *et al.*, 2008). The technique has great potential in the screening of tomato cultivars for disease resistance during breeding programs.

The two PCR based methods used in the analysis of the *Sw-5* gene utilised the same reagents except for the intercalating dye, Syto 9 that was added in the HRM analysis. However, the major difference between the two methods used was that PCR tests can be done with relatively simple equipment and can be seen clearly on a gel based on the amplicon sizes while HRM requires an expensive piece of equipment as well as software for data analysis. On the other hand, HRM is quicker and cheaper than the gel-based assay and has been reported to be more sensitive with ability to discriminate change arising from a single nucleotide and thus could be recommended for SNP analysis, particularly where change in amplicon size is uncertain (Distefano *et al.*, 2012; Wu *et al.*, 2008). Furthermore, the traditional SNP detection method is even more time consuming and expensive, and most importantly, less accurate (Wu *et al.*, 2008). In the current case there seems to be major differences between the resistant and susceptible cultivars that are detected with ordinary PCR with HRM being used in the identification of SNP.

Tomato cultivars showed different rates of thrips infestation under field conditions. Although there were variations in populations among the cultivars, there was no detectable field resistance to thrips that was observed among the tomato cultivars evaluated. Riley *et al.* (2011a) reported no significant difference in thrips population among tomato cultivars under field conditions with no detectable field resistance. Thrips occurring within tomato production areas contribute significantly to the spread of TSWV and therefore the ability of plants to resist thrips infestation is important in the management of TSWV (Leiss *et al.*, 2009; Maris *et al.*, 2002; Mirnezhad *et al.*, 2010). Maris *et al.* (2003b) observed that there was reduced spread of *Groundnut bud necrosis virus* in thrips-resistant groundnuts and TSWV infection was delayed on thrips-resistant pepper. The low population of thrips identified during the field trial could be attributed to unfavourable weather conditions, especially excess rainfall that was experienced during the trial period. Rainfall has been shown to affect thrips population as it leads to high mortality of larvae and suppression of flight (Morsello *et al.*, 2008; Morsello & Kennedy, 2009; Riley *et al.*, 2011a). Reduced recovery of up to six weeks was reported in *Frankliniella fusca* (Morsello & Kennedy, 2009) and this could significantly affect TSWV transmission. Further studies to establish the reaction of different cultivars to thrips infestation would be necessary.

In conclusion, the study has shown the occurrence of a limited number of cultivars resistant to TSWV despite the disease being endemic in the area where it was first reported to occur. This indicates there is need to create awareness on the importance of resistant cultivars in the management of the disease with a view to integrate resistance in tomato production in the region. However, the occurrence of resistance breaking races, possibility of persistence of the disease and heavy infestation with thrips, use of virus and thrips resistant plants alongside other control measures is advisable in the management of TSWV. The study

further PCR-based methods were used to effectively identify the occurrence of a resistance gene among the resistant cultivars and the analysis of SNPs using HRM was shown to be able to discriminate resistant or susceptible cultivars. Therefore, these results indicates PCR-based markers and gene-derived SNP markers can be important tool for identification *Sw-5* gene or alleles of tomato spotted wilt virus resistance research.

Chapter 7. General Discussion, Conclusion and Recommendations

This study was aimed at improving the understanding of the distribution and genetic diversity of TSWV a decade after its incursion into Kenya (Chapter 3). The study further evaluated the occurrence and genetic diversity of thrips as vectors (Chapter 4), weed species as alternative hosts of TSWV and its vectors (Chapter 5) and the presence of resistant cultivars (chapter 6) that are important in the management of the disease.

It was established that TSWV had persisted in the Nakuru area where it was first reported over a decade ago (Wangai *et al.*, 2001), but with limited distribution. Although the disease had persisted in this area, there was low awareness among farmers on the disease, its vectors and alternate hosts. The persistence of the disease could have been attributed to the presence of an effective vector (*F. occidentalis*) and suitable alternate host plants, including weed species as was observed in Chapters 4 and 5, respectively. Furthermore, due to the low awareness of the disease that was observed among the farmers, there was little effort to manage the disease and its vectors. The limited distribution indicates low natural dispersal potential but suggests the importance of infected materials or vectors in the spread of the disease. Since tomato is propagated through seeds there was no movement of tomato planting materials between the regions thereby leading to the limited distribution. The geographical separation between the production areas could also have limited the distribution. However, due to an established horticultural industry with significant movement of cut flowers and propagation materials in the country (EFSA, 2012; Jones, 2005), there is a risk of introduction of TSWV and its vectors. Therefore, there is a need for

further research to establish the occurrence of the disease and its vectors in other production systems.

It was observed that samples collected from Bungoma that had symptoms resembling those caused by *Tomato spotted wilt virus* tested negative to the virus and several other tospoviruses (Chapter 3). Furthermore, no known vectors were identified among thrips samples collected from this area (Chapter 4). *Tomato yellow ring spot virus* (TYRV) which causes symptoms similar to TSWV was reported in Kenya recently (Birithia *et al.*, 2012), TYRV was not detected by either ELISA or RT-PCR in this study. Although symptom expression has been used in the identification of the virus, TSWV has been shown to produce a wide range of symptoms, indicating that these are not reliable in its identification. The symptoms observed could have been due to a new strain of TSWV or to other viruses. Therefore, there is need to undertake further analysis using the next generation sequencing to establish the cause of the symptoms.

The genetic diversity of the Kenyan TSWV isolates was established through phylogenetic analysis which indicated the isolates clustered with isolates from Europe, Australia and South Africa but formed a single independent clade (Chapter 3). The limited sequence diversity among the Kenyan TSWV isolates suggests the virus was recently introduced but had undergone some local diversification. The study further revealed that, the Kenyan isolates were not closely related to the long-established South African population, suggesting an introduction from outside Africa, possibly from Europe. Therefore, the limited distribution of the virus in Kenya and the lack of evidence of spread from South Africa has implications for quarantine within the African continent.

Quarantine regulation and phytosanitary certification have been utilised to limit the introduction and spread of pests and diseases. However, despite the effort to develop appropriate quarantine systems in African countries, the capacity to diagnose virus diseases and their vectors is still lacking (Olembo, 2001). The current study employed a variety of approaches for identification of TSWV in tomato production. Serological and RT-PCR methods indicated their relative reliability in identification of TSWV. Immunostrips which are easy to use and require no equipment were found to be comparable to DAS-ELISA but were less sensitive than RT-PCR. On the other hand, DAS-ELISA was the cheapest method but had a relatively high risk of false negative results. Although RT-PCR was rated as the most expensive, it was the most sensitive and reliable method which could be used in the confirmation of the disease. This indicates there is need for further research to identify methods that are simple, sensitive and reliable to supplement the methods used in this study. Loop-mediated isothermal amplification (LAMP) has been shown to be simple, sensitive and reliable method for identification of virus, bacteria and fungi (Fukuta *et al.*, 2004; Goto *et al.*, 2009; Li & Ling, 2014). This method has potential to provide low cost reliable method for the identification of viruses including TSWV.

The cytochrome oxidase 1 (CO1) gene was used in the identification of thrips species where resulting sequences were compared with sequences in the GenBank and Barcode of Life Data Systems (BOLD). The phylogenetic analysis provided detailed information on the individual thrips species (Chapter 4). Use of the CO1 gene provided a simple, reliable and accurate method for the identification of a large number of samples compared to morphological identification which requires taxonomic expertise and is time consuming and laborious. The method also allowed for further analysis of the isolates and their comparison with isolates identified from other regions. Furthermore, the method was utilised in the

analysis of variation occurring within the different thrips species. The CO1 protocol that was utilised in the identification of thrips species in tomato production has the potential for routine identification of other insect pests and vectors.

A wide range of thrips species which included important vectors of TSWV were identified among samples collected from tomato production areas (Chapter 4). *F. occidentalis* and *T. tabaci* which have been reported as important vectors were among the most abundant thrips species in Nakuru, Kirinyaga and Loitokitok whereas *F. schultzei* and *Scirtothrips dorsalis* had low populations. Phylogenetic analysis indicated that *F. occidentalis* isolates collected from the production areas belonged to the western flower thrips 'glasshouse group' which has been shown to be more aggressive, and rapidly develops resistance to chemicals. Similarly, *T. tabaci* sequences formed three clusters consisting of tobacco, arrhenotokous and thelytokous groups. Although the Kenyan *T. tabaci* isolates clustered with the arrhenotokous and thelytokous groups, only the arrhenotokous group was identified from samples collected from the tomato production areas. The arrhenotokous group is considered important in TSWV transmission (Cabrera-La Rosa & Kennedy, 2007; Chatzivassiliou *et al.*, 2002).

F. schultzei which is one of the known vectors was found to be the most diverse group and could consist of different species. The species exists as the dark form and the pale form and have been placed in a single polymorphic species due to lack of morphological differences (Sakimura, 1969). The dark form is considered to be more efficient in the transmission of tospoviruses than the pale form. Unlike *F. occidentalis* and *T. tabaci*, *F. schultzei* was less common in the surveyed areas, hence is not likely to play a significant role in the

epidemiology of TSWV in Kenya. However, there is need for further work to discriminate the two forms and to evaluate their transmission potential.

On the other hand, *C. brunneus* was the most abundant thrips species in all the tomato production areas. *Ceratothripoides brunneus* is native to Africa but has recently been identified in other countries including Malaysia, Puerto Rico, and the Netherlands (Mound & Azidah, 2009; Mound & Nickle, 2009). Although *C. brunneus* has been associated with yield losses in tomatoes, there is no report on its status as a vector of tospoviruses. In this genus, only *C. clarastris* has been identified as a vector of tospoviruses, specifically *Capsicum chlorosis virus* (Steenken & Halawel, 2011). Since *C. brunneus* was the most abundant and widely distributed species within all the production areas, there is need for further research to establish its possible role as a vector.

The ability of TSWV to infect a wide range of plant species has implications in the management of the virus. Successful management of the TSWV is based on understanding the source of the inoculum within the production systems. A survey of weeds indicated occurrence of a wide range of weed species in tomato production, most of which had previously been reported as important hosts of TSWV (Chapter 5). Although weed species that are susceptible to the virus are important as a reservoir for the inoculum, species that are susceptible to the virus and supports thrips reproduction have been show to be important in disease epidemiology. Therefore, management of *A. hybridus, D. stramonium, T. minuta,* and *S. nigrum* which were susceptible to TSWV and supported high thrips reproduction was viewed as important in the effort to control the virus within tomato production. On the other hand, *Commelina cyanea* and *O. latifolia*, which were recorded as new hosts of the virus,

supported low thrips reproduction but occurred frequently within the production areas should not be ignored as long-term survival hosts for the virus.

The reproduction potential of *F. occidentalis* which is known to infect a wide range of plant species, was established on a variety of weed species (Chapter 5). Although transmission and reproduction was observed on selected weed species, *G. parviflora* and *S. oleraceus* supported thrips reproduction, but no transmission through *F. occidentalis* was recorded. On the other hand, *T. tabaci* has been reported to infest several weed species but there is no clear status on the range of weed species infested by *F. schultzei*. Collection of viruliferous *T. tabaci* from *S. oleraceus* in tobacco production indicated their important role in the transmission of TSWV (Chatzivassiliou *et al.*, 2007); however, the inability to transmit the virus *S. oleraceus* in the current study together with high larval mortality repeated in previous studies suggests the need to evaluate their transmission potential. The ability of the weeds to support reproduction of vectors has been demonstrated as important in determining the vector competence as well their role in disease epidemiology. Therefore there is need to evaluate the ability of the weeds to support reproduction of other vectors and establish their potential in the transmission of the virus.

The variation observed in the ability of weeds to support the reproduction of *F. occidentalis* in this study could have been influenced by the nutritional quality of the host plants and the presence or absence of plant defence compounds (Delphia *et al.*, 2007; Koschier *et al.*, 2007). Shrestha *et al.* (2012) observed that there was a correlation between the amount of nitrogen, amino acid and sugars with thrips oviposition and the number of feeding scars that indicated the level of feeding (Shrestha *et al.*, 2012). Phenolic compounds on the other hand have been repoeted to protect the plants against insect infestation by altering the nutritional

value and insect preference thereby affecting the feeding and oviposition by thrips (Bi *et al.*, 1997; Papadaki *et al.*, 2008). However, there is limited information on the chemical composition of most weeds and their possible role in the thrips ovipostion and feed. Thus, there is need to understand the chemical composition of the different weed species and their effect on thrips preference and reproduction potential.

Plant resistance is widely used in the management of TSWV. Evaluation for resistance indicated a varied reaction of tomato cultivars to TSWV infection (Chapter 6). Among the commercial cultivars that were evaluated, only a few were reported to be resistant to TWSV and contained the *Sw-5* gene. However, some cultivar showed low infection to TSWV with some not showing any infection. The varieties resistant to TSWV can be recommended to replace the popular variety Rio Grande in Nakuru where TSWV was identified. However, there is need for further evaluation to establish the status of the cultivar that showed low infection under field condition and possibility of existence of difference resistance gene. Furthermore, owing to the occurrence of resistance breaking races and the possibility of persistence and heavy infestation with thrips which have complicated the management of the disease, use of virus and thrips-resistant plants alongside other management measures is advisable in the management of TSWV. Therefore there is need for research on thrips resistance as an alternative strategy in the management of the virus alongside research on additional resistant genes forTSWV.

This study has revealed that the disease has persisted where it was first reported more than a decade ago and has limited distribution. This represents a comprehensive study on the status of TSWV and its vectors in Kenya and to a larger extent in the East African region. The findings reiterate the importance of the disease in areas where it was first reported and the

significant role of vectors and alternate hosts in the persistence of the disease in this production area. The presence of alternate host indicates a ready sink for inoculum as they act as a reservoir for the virus and the vectors. The presence of vectors and susceptible hosts in areas where the disease has not been reported also indicates the potential risk in the event that the disease is introduced. The variation observed within the vector populations was important in understanding the role of the vector species and implications for their management. Thus future research on the management of TSWV should take into account the genetic variation within the thrips species and the susceptibility of the host plants.

Furthermore, the current study employed a variety of approaches to understand TSWV in the Kenyan tomato production areas. The approaches have identified key factors that are important in the epidemiology of TSWV and which should be considered in the development of appropriate management systems. Although the approaches provided substantial information about TSWV, there is need for further work to understand the occurrence of other tospoviruses in Kenya and other African countries. The competence of *F. occidentalis* in the transmission of TSWV to weed species has supported their role in the disease cycle and laid a foundation for further research on the transmission studies. Furthermore, the experience learnt is important in the development of appropriate identification and management strategies for viral diseases in tomato production systems.

References

Abad, J., A., Moyer, J. W., Kennedy, G. G., Gerald, A., & Cubeta, M. A. (2005). *Tomato spotted wilt virus* on potato in eastern North Carolina. *American Journal of Potato Reserach, 82*, 255–261.

Adkins, S. (2000). *Tomato spotted wilt virus*-positive steps towards negative success. *Molecular Plant Pathology, 2*, 51–157. doi: 10.1046/j.1364-3703.2000.00022.x

Adkins, S., & Rosskopf, E. N. (2002). Key west nightshade, a new experimental host for plant viruses. *Plant Disease, 86*, 1310–1314.

Allen, W. R., & Matteoni, J. A. (1991). Petunia as an indicator plants for use by growers to monitor for thrips carrying the tomato spotted wilt virus in greenhouses. *Plant Disease, 75*, 78–82.

Aramburu, J., Galipienso, L., Soler, S., & Lopez, C. (2010). Characterisation of *Tomato spotted wilt virus* isolate that overcomes the *Sw-5* resistant gene in tomato and fitness assays. *Phytopathologia Mediterranea, 49*, 342–351.

Aramburu, J., & Marti, M. (2003). The occurrence in north-east Spain of a variant of *Tomato spotted wilt virus* (TSWV) that breaks resistance in tomato (*Lycopersicon esculentum*) containing the *Sw-5* gene. *Plant Pathology, 52*, 407.

Aramburu, J., Rodriguez, M., & Arino, J. (2000). Effect of Tomato spotted wilt tospovirus (TSWV) infection on the fruits of tomato (*Lycopersicon esculentum*) plants of cultivars carrying the *Sw-5* gene. *Journal of Phytopathology 148*, 569–574.

Assis Filho, F. M. de., Deom, C. M., & Sherwood, J. L. (2004). Acquisation of *Tomato spotted wilt virus* (TSWV) by adults of two thrips species. *Phytopathology, 94*, 333–336.

Assis Filho, F. M. de., Naidu, R. A., Deom, C. M., & Sherwood, J. L. (2002). The dynamics of TSWV replication in the alimentary canal of two thrips species. *Phytopathology, 92*, 729–733.

Atakan, E., Kamberoğlu, M. A., & Uygur, S. (2013). Role of weed hosts and the western flower thrips, *Frankliniella occidentalis*, in epidemiology of *Tomato spotted wilt virus* in the Çukurova region of Turkey. *Phytoparasitica, 41*(5), 577–590. doi: 10.1007/s12600-013-0318-9

Avila, Y., Stavisky, J., Hague, S., Funderburk, J., Reitz, S., & Momol, T. (2006). Evaluation of *Frankliniella bispinosa* (Thysanoptera: Thripidae) as a vector of the *Tomato spotted wilt virus* in pepper. *Florida Entomologist, 89*(2), 204–207.

Bautista, R. C., Mau, R. F. L., Cho, J. J., & Custer, D. M. (1995). Potential of tomato spotted wilt tospovirus plant hosts in Hawaii as reservoir for transmission of *Frankliniella occidentalis* (Thysanoptera: Thripidae). *Phytopathology, 85*, 953–958.

Bi, J. L., Murphy, J. B., & Felton, G. W. (1997). Does salicylic acid act as a signal in cotton for induced resistance to *Helicoverpa zea*. *Journal of Chemical Ecology, 23*(7), 1805–1818.

Birithia, R., Subramanian, S., Pappu, H. R., Sseruwagi, P., Muthomi, J. W., & Narla, R. D. (2011). First report of *Iris yellow spot virus* infecting onion in Kenya and Uganda. *Plant Disease 95*(9), 1195.

Birithia, R., Subramanian, S., Villinger, J., Muthomi, J. W., Narla, R. D., & Pappu, H. R. (2012). First report of *Tomato yellow ring virus* (*Tospovirus*, Bunyaviridae) infecting tomato in Kenya. *Plant Disease, 96*(9), 1384–1384. doi: 10.1094/pdis-05-12-0462-pdn

Boiteux, L. S., Nagata, T., Dutra, W. E., & Fonseca, M. E. N. (1993). Sources of resistance to *Tomato spotted wilt virus* (TSWV) in cultivated and wild species of *Capsicum*. *Euphytica, 67*, 89–94.

Boonham, N., Smith, P., Walsh, K., Tame, J., Morris, J., Spence, N., Bennison, J., & Barker, I. (2002). The detection of *Tomato spotted wilt virus* (TSWV) in individual thrips using real time fluorescent RT-PCR (TaqMan). *Journal of Virological Methods, 101*, 37–48.

Brittlebank, C. C. (1919). Tomato diseases. *Journal of Agriculture Victoria, 27*, 231–235.

Brødsgaard, H. F. (1994). Insecticide resistance in European and African strains of western flower thrips (Thysanoptera; Thripidae) tested in a new residue-on-glass test. *Journal Econonomic Entomology, 87*, 1141–1146.

Brommonschenkel, S. H., Frary, A., Frary, A., & Tanksley, S. D. (2000). The broad-spectrum tospovirus resistance gene *Sw-5* of tomato is a homolog of the root-knot nematode resistance gene Mi. *Molecular Plant-Microbe Interactions, 13*(10), 1130–1138.

Brunner, P. C., Chatzivassiliou, E. K., Katis, N. I., & Frey, J. E. (2004). Host-associated genetic differentiation in *Thrips tabaci* (Insecta; Thysanoptera), as determined from mtDNA sequence data. *Heredity 93*(4), 364–370. doi: 10.1038/sj.hdy.6800512

Cabrera-La Rosa, J. C., & Kennedy, G. G. (2007). *Thrips tabaci* and *Tomato spotted wilt virus*: inheritance of vector competence. *Entomologia Experimentalis et Applicata, 124*(2), 161–166. doi: 10.1111/j.1570-7458.2007.00564.x

Caicedo, A. L., Williamson, S. H., & Hernandez, R. D. (2007). Genome-wide patterns of nucleotide polymorphism in domesticated rice. *PLoS Genetics, 3*, 1745–1756. doi: 10.1371/journal.pgen.0030163.eor

Campbell, J. K., Nanene-Adams, K., Lindshield, B. L., Boileau, T. W. M., Clinton, S. K., & Erdman, J. W. (2004). Tomato phytochemicals and prostrate cancer risk. *Journal of Nutrition, 134*, 3486–3492.

Canady, M. A., Stevens, M. R., Barineau, M. S., & Scott, J. W. (2001). *Tomato spotted wilt virus* (TSWV) resistance in tomato derived from *Lycopersicon chilense* Dun. LA 1938. *Euphytica 117*, 19–25.

Cebolla-Cornejo, J., Soler, S., Gomar, B., Soria, M. D., & Nuez, F. (2003). Screening Capsicum germplasm for resistance to *Tomato spotted wilt virus* (TSWV). *Annals of Applied Biology 143*, 143–152.

Chatzivassiliou, E. K., Boubourakas, I., Drossos, E., Eleftherohorinos, I., Jenser, G., Peters, D., & Katis, N. I. (2001). Weeds in greenhouses and tobacco fields are differentially infected by *Tomato spotted wilt virus* and infested by its vector species. *Plant Disease 85*, 40–46.

Chatzivassiliou, E. K., Nagata, T., Katis, N. I., & Peters, D. (1999). Transmission of tomato spotted wilt tospovirus by *Thrips tabaci* population originating from leeks. *Plant Pathology, 48*, 700–706.

Chatzivassiliou, E. K., Peters, D., & Katis, N. I. (2002). The efficiency by which *Thrips tabaci* populations transmit *Tomato spotted wilt virus* depends on their host preference and reproductive strategy. *Phytopathology, 92*, 603–609.

Chatzivassiliou, E. K., Peters, D., & Katis, N. I. (2007). The role of weeds in the spread of Tomato spotted wilt virus by *Thrips tabaci* (Thysanoptera: Thripidae) in tobacco crops. *Journal of Phytopathology, 155*(11-12), 699–705. doi: 10.1111/j.1439-0434.2007.01302.x

Chatzivassiliou, E. K., Weekes, R., Morris, J., Wood, K. R., Barker, I., & Katis, N. I. (2000). *Tomato spotted wilt virus* (TSWV) in Greece: Its incidence following the expansion of *Frankliniella occidentalis*, and characterisation of isolates collected from various hosts. *Annals of Applied Biology, 137*, 127–134.

Chen, T.-C., Lu, Y.-Y., Cheng, Y.-H., Li, J.-T., Yeh, Y.-C., Kang, Y.-C., Chang, C.-P., Huang, L.-H., Peng, J.-C., & Yeh, S.-D. (2010). Serological relationship between Melon yellow spot virus and Watermelon silver mottle virus and differential detection of the two viruses in cucurbits. *Archives of Virology, 155*(7), 1085–1095. doi: 10.1007/s00705-010-0688-y

Chen, T. C., Li, J. T., Lin, Y. P., Yeh, Y. C., Kang, Y. C., Huang, L. H., & Yeh, S. D. (2012). Genomic characterization of Calla lily chlorotic spot virus and design of broad spectrum primers for detection of tospoviruses. *Plant Pathology 61*, 183–194. doi: 10.1111/j.1365-3059.2011.02484.x

Cheng, Y. H., Zheng, Y. X., Tai, C. H., Yen, J. H., Chen, Y. K., & Jan, F. J. (2014). Identification, characterisation and detection of a new tospovirus on sweet pepper. *Annals of Applied Biology, 164*(1), 107–115. doi: 10.1111/aab.12084

Chiemsombat, P., Gajanandana, O., Warin, N., Hongprayoon, R., Bhunchoth, A., & Pongsapich, P. (2008). Biological and molecular characterization of tospoviruses in Thailand. *Archives of Virology, 153*(3), 571–577. doi: 10.1007/s00705-007-0024-3

Cho, J. J., Custer, D. M., Brommonschenkel, S. H., & Tanksley, S. D. (1996). Conventional breeding: Host-plant resistance and the use of molecular markers to develop resistance to Tomato spot wilt virus in vegetables. *Acta Horticulturae, 431*, 367–378.

Cho, J. J., Mau, R. F., German, T. L., Hartmann, R. W., & Yudin, L. S. (1989). A multidisciplinary approach in the management of *Tomato spotted wilt virus* in Hawaii. *Plant Disease, 73*, 375–383.

Cho, J. J., Mau, R. F. L., Gonsalves, D., & Mitchel, W. C. (1986). Reservoir weeds host of *Tomato spotted wilt virus*. *Plant Disease, 70*, 1014–1017.

Cho, J. J., Mau, R. F. L., Hamasaki, R. T., & Gonsalves, D. (1988). Dectection of tomato spotted wilt virus in individual thrips species by enzyme-linked immunosorbent assay. *Phytopathology, 78*, 1348–1352.

Cho, J. J., Mau, R. F. L., Mitchell, W. C., Gonsalves, D., & Yudin, L. S. (1987). Host list of plants susceptible to *Tomato spotted wilt virus* (TSWV) (Research Series 078, pp. 1–10): University of Hawaii. http://hdl.handle.net/10125/5893.

Chu, F. H., Chao, C. H., Chung, M. H., Chen, C. C., & Yeh, S. D. (2001). Completion of the genome sequence of Watermelon silver mottle virus and utilization of degenerate primers for detecting tospoviruses in five serogroups. *Phytopathology, 91*, 361–368.

Ciuffo, M., Finetti-Sialer, M. M., Gallitelli, D., & Turina, M. (2005). First report in Italy of a resistance-breaking strain of *Tomato spotted wilt virus* infecting tomato cultivars carrying the *Sw-5* resistance gene. *Plant Pathology, 54*, 564.

Ciuffo, M., Kurowski, C., Vivoda, E., Copes, B., Masenga, V., Falk, B. W., & Turina, M. (2009). A new *Tospovirus* in cucurbit crops in Mexico. *Plant Disease, 93*(5), 467–474. doi: 10.1094/pdis-93-5-0467

Ciuffo, M., Mautino, G. C., Bosco, L., Turina, M., & Tavella, L. (2010). Identification of *Dictyothrips betae* as the vector of Polygonum ring spot virus. *Annals of Applied Biology, 157*(2), 299–307. doi: 10.1111/j.1744-7348.2010.00428.x

Ciuffo, M., Tavella, L., Pacifico, D., Masenga, V., & Turina, M. (2008). A member of a new Tospovirus species isolated in Italy from wild buckwheat (*Polygonum convolvulus*). *Archives of Virology, 153*(11), 2059–2068. doi: 10.1007/s00705-008-0228-1

Culbreath, A. K., & Srinivasan, R. (2011). Epidemiology of spotted wilt disease of peanut caused by Tomato spotted wilt virus in the southeastern U.S. *Virus Research, 159*, 101–109. doi: 10.1016/j.virusres.2011.04.014

Dafalla, G. A. (2001). *Situation of tomato and pepper viruses in Africa.* Paper presented at the Plant virology in Sub-Saharan Africa International Institute of Torical Agriculture, Ibadan, Nigeria.

Dang, P. M., Rowland, D., & Faircloth, W. H. (2009). Comparison of ELISA and RT-PCR assays for the detection of *Tomato spotted wilt virus* (TSWV) infection in peanut. *Peanut Science, 36*, 133–137.

de Avila, A. C., de Haan, P., Kormelink, R., Resende, R. de O., Goldbach, R. W., & Peters, D. (1993). Classification of tospoviruses based on phylogeny of nucleoprotein gene sequences. *Journal of General Virology, 74*, 153–159.

de Borbón, C. M., & Gracia, O. (1996). *Frankliniella schultzei* (Trybom), eficiente vector de *groundnut ringspot tospovirus* (GRSV) en cultivos de lechuga en Mendoza, Argentina. (suplemento). *Fitopatología Brasileira, 21*, 423.

de Borbón, C. M., Gracia, O., & Píccolo, R. (2006). Relationships between Tospovirus incidence and thrips populations on tomato in Mendoza, Argentina. *Journal of Phytopathology, 154*(2), 93–99. doi: 10.1111/j.1439-0434.2006.01066.x

de Haan, P., Wagemakers, L., Peters, D., & Goldbach, R. (1990). The S RNA segment of tomato spotted wilt virus has an ambisense character. *Journal of General Virology 71*, 1001–1007.

de Oliveira, A. S., Melo, F. L., Inoue-Nagata, A. K., Nagata, T., Kitajima, E. W., & Resende, R. O. (2012). Characterization of Bean necrotic mosaic virus: A member of a novel evolutionary lineage within the genus Tospovirus. *PLoS ONE, 7*(6), e38634. doi: 10.1371/journal.pone.0038634

Debreczeni, D. E., Ruiz-Ruiz, S., Aramburu, J., Lopez, C., Belliure, B., Galipienso, L., Soler, S., & Rubio, L. (2011). Detection, discrimination and absolute quantitation of *Tomato spotted wilt virus* isolates using real time RT-PCR with TaqMan MGB probes. *Journal of Virological Methods, 176*(1-2), 32–37. doi: 10.1016/j.jviromet.2011.05.027

DeGraaf, H. E., & Wood, G. M. (2009). An improved method for rearing western flower thrips *Frankliniella occidentalis*. *Florida Entomologist, 92*(4), 664–666. doi: 10.1653/024.092.0424

Delphia, C. M., Mescher, M. C., & De Moraes, C. M. (2007). Induction of plant volatiles by herbivores with different feeding habits and the effects of induced defenses on host-plant selection by thrips. *Journal of Chemical Ecology, 3*, 997–1012.

Dianese, É. C., Fonseca, M. E. N., Goldbach, R., Kormelink, R., Inoue-Nagata, A. K., Resende, R. O., & Boiteux, L. S. (2010). Development of a locus-specific, co-dominant SCAR marker for assisted-selection of the *Sw-5* (*Tospovirus* resistance) gene cluster in a wide range of tomato accessions. *Molecular Breeding, 25*(1), 133–142. doi: 10.1007/s11032-009-9313-8

Dianese, E. C., Fonseca, M. E. N., Inoue-Nagata, A. K., Resende, R. O., & Boiteux, L. S. (2011). Search in *Solanum* (section *Lycopersicon*) germplasm for sources of broad-spectrum resistance to four *Tospovirus* species. *Euphytica, 180*(3), 307–319. doi: 10.1007/s10681-011-0355-8

Dietzgen, R. G., Twin, J., Talty, J., Selladurai, S., Carroll, M. L., Coutts, B. A., Berryman, D. I., & Jones, R. A. C. (2005). Genetic variability of *Tomato spotted wilt virus* in Australia and validation of real time RT-PCR for its detection in single and bulked leaf samples. *Annals of Applied Biology, 146*(4), 517–530. doi: 10.1111/j.1744-7348.2005.040155.x

Distefano, G., Caruso, M., La Malfa, S., Gentile, A., & Wu, S. B. (2012). High resolution melting analysis is a more sensitive and effective alternative to gel-based platforms in analysis of SSR-an example in citrus. *PLoS ONE, 7*(8), e44202. doi: 10.1371/journal.pone.0044202

Distefano, G., La Malfa, S., Gentile, A., & Wu, S.-B. (2013). EST-SNP genotyping of citrus species using high-resolution melting curve analysis. *Tree Genetics & Genomes, 9*(5), 1271–1281. doi: 10.1007/s11295-013-0636-6

Dong, J.-H., Cheng, X.-F., Yin, Y.-Y., Fang, Q., Ding, M., Li, T.-T., Zhang, L.-Z., Su, X.-X., McBeath, J., & Zhang, Z.-K. (2008). Characterization of tomato zonate spot virus, a new *Tospovirus* in China. *Archives of Virology, 153*(5), 855–864. doi: 10.1007/s00705-008-0054-5

du Toit L. J., Burger, J. T., McLeod, A., Engelbrecht, M., & Viljoen, A. (2007). Iris yellow spot virus in onion seed crops in South Africa. *Plant Disease 91* (9), 1203.

Duffus, J. E. (1971). Role of weeds in the incidence of virus diseases. *Annual Review of Phytopathology, 9*, 319–340.

EFSA. (2012). Scientific opinion on the risk to plant health posed by *Tomato spotted wilt virus* to the EU territory with identification and evaluation of risk reduction options. *EFSA Journal, 10*(7), 2772.

EPPO. (2002). *Frankliniella occidentalis*. *EPPO Bulletin, 32*(2), 281–292. doi: 10.1046/j.1365-2338.2002.00584.x

EPPO. (2014). *PQR - EPPO database on quarantine pests* (online). URL https://www.eppo.int/DATABASES/pqr/pqr.htm [Accessed 23 February 2015].

FAO. (2012). *Agricultural statistics for the year 2012* URL http://faostat3.fao.org/download/Q/QC/E [Accessed 20 March 2015].

Foolad, M. R. (2007). Genome mapping and molecular breeding of tomato. *International Journal of Plant Genomics, 2007*, 64358. doi: 10.1155/2007/64358

Fukuta, S., Ohishi, K., Yoshida, K., Mizukami, Y., Ishida, A., & Kanbe, M. (2004). Development of immunocapture reverse transcription loop-mediated isothermal amplification for the detection of *Tomato spotted wilt virus* from chrysanthemum. *Journal of Virological Methods, 121*(1), 49–55. doi: 10.1016/j.jviromet.2004.05.016

Gao, Y., Lei, Z., & Reitz, S. R. (2012). Western flower thrips resistance to insecticides: Detection, mechanisms, and management strategies. *Pest Management Science, 68*, 1111–1121. doi: 10.1002/ps.3305

Garland, S., Sharman, M., Persley, D., & McGrath, D. (2005). The development of an improved PCR-based marker system for *Sw-5*, an important TSWV resistance gene of tomato. *Australian Journal of Agricultural Research, 56*(3), 285. doi: 10.1071/ar04140

Gera, A., Kritzman, A., Cohen, J., Raccah, B., & Antignus, Y. (2000). Tospoviruses infecting vegetable crops in Israel. *OEPP/EPPO Bulletin, 30*, 289–292.

German, T. L., Ullman, D. E., & Moyer, J. W. (1992). Tospoviruses: Diagnosis, molecular biology, phylogeny, and vector relationships. *Annual Reveiw of Phytopathology, 30*, 315–348.

Giancola, S., McKhann, H. I., Be´rard, A. l., Camilleri, C., Durand, S. p., Libeau, P., Roux, F., Reboud, X., Gut, I. G., & Brunel, D. (2006). Utilization of the three high-throughput SNP genotyping methods, the GOOD assay, Amplifluor and TaqMan, in diploid and polyploid plants. *Theoretical and Applied Genetics, 112*, 1115–1124. doi: 10.1007/s00122-0060213-6

Giordano, L. B., Boiteux, L. S., Quezado-Duval, A. M., Fonseca, M. E. N., Resende, F. V., Reis, A., González, M., Nascimento, W. M., & Mendonça, J. L. (2010). 'BRS Tospodoro': A high lycopene processing tomato cultivar adapted to organic cropping systems and with multiple resistance to pathogens. *Horticultura Brasileira 28*, 241–245.

Giordano, L. B., de Avila, A. C., Charchar, J. M., Boiteux, L. S., & Ferraz, E. (2000). 'Viradoro': A tospovirus-resistant processing tomato cultivar adapted to tropical environments *HortScience, 35*, 1368–1370.

Giovannucci, E. (1999). Tomatoes, tomato-based products, lycopene, and cancer: Review of epidemiologic literature. *Journal of the National Cancer Institute, 91*(4), 317–331. doi: 10.1093/jnci/91.4.317

Golnaraghi, A. R., Pourrahim, R., Farzadfar, S., Ohshima, K., Shah-raeen, N., & Ahoonmanesh, A. (2007). Incidence and distribution of Tomato yellow fruit ring virus on soybean in Iran. *Journal of Plant Pathology 6*, 14–21.

Gordillo, L. F., Stevens, M. R., Millard, M. A., & Geary, B. (2008). Screening two *Lycopersicon peruvianum* collections for resistance to*Tomato spotted wilt virus*. *Plant Disease, 92*(5), 694–704. doi: 10.1094/pdis-92-5-0694

Goto, M., Honda, E., Ogura, A., Nomoto, A., & Hanaki, K. (2009). Colorimetric detection of loop-mediated isothermal amplification reaction by using hydroxy naphthol blue. *Biotechniques, 46*(3), 167–172. doi: 10.2144/000113072

Gracia, O., de Borron, C. M., de Millan, G. N., & Cuesta, G. V. (1999). Occurrence of different tospoviruses in vegetable crops in Argentina. *Journal of Phytopathology, 147*, 223–227.

Groves, R. L., Walgenbach, J. F., Moyer, J. W., & Kennedy, G. G. (2002). The role of weed hosts and tobacco thrips, *Frankliniella fusca*, in the epidemiology of *Tomato spotted wilt virus*. *Plant Disease, 86*, 573–582.

Grund, E., Darissa, O., & Adam, G. (2010). Application of FTA® cards to sample microbial plant pathogens for PCR and RT-PCR. *Journal of Phytopathology, 158*(11-12), 750–757. doi: 10.1111/j.1439-0434.2010.01695.x

Hagen, C., Frizzi, A., Kao, J., Jia, L., Huang, M., Zhang, Y., & Huang, S. (2011). Using small RNA sequences to diagnose, sequence, and investigate the infectivity characteristics of vegetable-infecting viruses. *Archives of Virology, 156*(7), 1209–1216. doi: 10.1007/s00705-011-0979-y

Hanssen, I. M., Lapidot, M., & Thomma, B. P. H. J. (2010). Emerging viral diseases of tomato crops. *Molecular Plant-Microbe Interation 23*(5), 539–548. doi: 10.1094/MPMI -23-5-0539

Hassani-Mehraban, A., Botermans, M., Verhoeven, J., Meekes, E., Saaijer, J., Peters, D., Goldbach, R., & Kormelink, R. (2010). A distinct tospovirus causing necrotic streak on Alstroemeria sp. in Colombia. *Archives of Virology, 155*(3), 423–428. doi: 10.1007/s00705-010-0590-7

Hassani-Mehraban, A., Cheewachaiwit, S., Relevante, C., Kormelink, R., & Peters, D. (2011). Tomato necrotic ring virus (TNRV), a recently described tospovirus species infecting tomato and pepper in Thailand. *European Journal of Plant Pathology, 130*(4), 449–456. doi: 10.1007/s10658-011-9771-9

Hebert, P. D. N., Cywinska, A., Ball, S. L., & de Waard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society B 270*, 313–321. doi: 10.1098/rspb.2002.2218

Herbert, D. A., Malone, S., Aref, S., Brandenburg, R. L., Jordan, D. L., Royals, B. M., & Johnson, P. D. (2007). Role of insecticides in reducing thrips injury to plants and incidence of Tomato spotted wilt virus in Virginia market-type peanut. *Journal of Economic Entomology, 100*(4), 1241–1247. doi: 10.1603/0022-0493(2007)100[1241:ROIIRT]2.0.CO

Hillocks, R. J. (2012). Farming with fewer pesticides: EU pesticide review and resulting challenges for UK agriculture. *Crop Protection, 31*(1), 85–93. doi: 10.1016/j.cropro.2011.08.008

Huang, K. S., Lee, S. E., Yeh, Y., Shen, G. S., Mei, E., & Chang, C. M. (2010). Taqman real-time quantitative PCR for identification of western flower thrip (*Frankliniella*

occidentalis) for plant quarantine. *Biology Letters, 6*(4), 555–557. doi: 10.1098/rsbl.2009.1060

ICTV. (2014). *International Committee on Taxonomy of Viruses (ICTV) 2014 master species list (MSL29)* http://talk.ictvonline.org/files/ictv_documents/m/msl/5208.aspx [Acessed 02 April 2015].

Immaranju, J. A., Paine, T. D., Bethke, J. A., Robb, K. L., & Newman, J. P. (1992). Western flower thrip (Thysanoptera: Thripidae) resistance to insectides in coastal California. *Journal of Entomology, 85*, 9–14.

Inoue, T., Sakurai, T., Murai, T., & Menda, T. (2004). Specificity of accumulation and transmission of *Tomato spotted wilt virus* (TSWV) in two genera, *Frankiniella* and *Thrips* (Thysanoptera:Thripidae). *Bulletin of Entomological Research, 94*, 501–507.

Jacobson, A. L., Booth, W., Vargo, E. L., & Kennedy, G. G. (2013). *Thrips tabaci* population genetic structure and polyploidy in relation to competency as a vector of tomato spotted wilt virus. *PLoS One, 8*(1), e54484. doi: 10.1371/journal.pone.0054484

Jacobson, A. L., & Kennedy, G. G. (2013). Specific insect-virus interactions are responsible for variation in competency of different *Thrips tabaci* isolines to transmit different *Tomato spotted wilt virus* isolates. *PLoS One, 8*(1), e54567. doi: 10.1371/journal.pone.0054567

Jensen, S. E. (2000). Insecticide resistance in the western flower thrips, *Frankliniella occidentalis*. *Integrated Pest Management Reviews 5*, 131-146.

Jenser, G., Gáborjányi, R., Szénási, A., Almási, A., & Grasselli, M. (2003). Significance of hibernated *Thrips tabaci* Lindeman (Thysan., Thripidae) adults in the epidemic of *Tomato spotted wilt virus*. *Journal of Applied Entomology, 127*(1), 7–11. doi: 10.1046/j.1439-0418.2003.00704.x

Johansen, R. M. (2002). The Mexican *Frankliniella fusca* (Hinds), *F. pallida* (Uzel) and *F. schultzei* (Trybom) species assemblages, in the 'Intonsa group' (Insecta, Thysanoptera, Thripidae). *Acta Zoológica Mexicana, 85*, 51–82.

Johnson, R. R., Black, L. L., Hobbs, H. A., & Valverde, R. A. (1995). Association of *Frankliniella fusca* and three winter weeds with *Tomato spotted wilt virus* in Louisiana. *Plant Disease, 79*, 572–576.

Jones, D. R. (2005). Plant viruses transmitted by thrips. *European Journal of Plant Pathology, 113*(2), 119–157. doi: 10.1007/s10658-005-2334-1

Jones, J. B., Stall, R. E., & Zitter, T. A. (1997). *Compedium of tomato diseases*. Minnesota USA: American Phytopathological Society Press.

Jones, R. A. (2004). Using epidemiological information to develop effective integrated virus disease management strategies. *Virus Research, 100*(1), 5–30. doi: 10.1016/j.virusres.2003.12.011

Kadirvel, P., Srinivasan, R., Hsu, Y.-C., Su, F.-C., & Peña, R. D. L. (2013). Application of cytochrome oxidase I sequences for phylogenetic analysis and identification of thrips species occurring on vegetable crops. *Journal of Economic Entomology, 106*(1), 408–418. doi: 10.1603/ec12119

Kahn, N. D., Walgenbach, J. F., & Kennedy, G. G. (2005). Summer weeds as hosts for *Frankliniella occidentalis* and *Frankliniella fusca* (Thysanoptera: Thripidae) and as reservoirs for Tomato spotted wilt tospovirus in North Carolina. *Journal of Economic Entomology, 98*(6), 1810–1815. doi: 10.1603/0022-0493-98.6.1810

Karimi, J., Hassani-Kakhki, M., & Awal, M. M. (2010). Identifying thrips (Insecta: Thysanoptera) using DNA barcodes. *Journal of Cell and Molecular Research, 2*(1), 35–41.

Kasina, M., Nderitu, J., Nyamasyo, G., Waturu, C., Olubayo, F., Obudho, E., & Yobera, D. (2009). Within-plant distribution and seasonal population dynamics of flower thrips (Thysanoptera: Thripidae) infesting French beans (*Phaseolus vulgaris* L.) in Kenya. *Spanish Journal of Agricultural Research 7*(3), 652–659.

Kedera, C., & Kuria, B. (2003). *Invasive alien species in Kenya: Identification of risks and management of invasive alien species using the IPPC framework*. Paper presented at the Invasive Alien Species and the International Plant Protection Convention, Braunschweig, Germany.

Kimenju, J. W., Karanja, N. K., & Nyongesa, M. W. (2004). Diversity and abundance of nematodes in agroecosystems of Kenya. *Journal of Tropical Microbiology, 3*, 24-33.

Kirk, W. D. J., & Terry, L. I. (2003). The spread of the western flower thrips *Frankliniella occidentalis* (Pergande). *Agricultural and Forest Entomology 5*, 301–310.

Knapp, M., Van Houten, Y., Hoogerbrugge, H., & Bolckmans, K. (2013). *Amblydromalus limonicus* (Acari: Phytoseiidae) as a biocontrol agent: Literature review and new findings. *Acarologia 53*(2), 191–202. doi: 10.1051/acarologia/20132088

Kobayashi, K., & Eisuke, H. (2012). Discrimination of reproductive forms of *Thrips tabaci* (Thysanoptera: Thripidae) by PCR with sequence specific primers. *Journal of Economic Entomology, 105*(2), 555–559. doi: doi.org/10.1603/EC11320

Kormelink, R., Garcia, M. L., Goodin, M., Sasaya, T., & Haenni, A.-L. (2011). Negative-strand RNA viruses: The plant-infecting counterparts. *Virus Research, 162*(1-2), 184–202.

Koschier, E. H., Hoffmann, D., & Reiner, J. (2007). Influence of salicylaldehyde and methylsalicylate on post-landing behaviour of *Frankliniella occidentalis* (Pergande). *Journal of Applied Entomology 131*, 362–367.

Kresta, K. K., Mitchell, F. L., & Smith, J. W. (1995). Survey by ELISA of thrips (Thysanoptera: Thripidae) vectored tomato spotted wilt virus distribution in foliage and flowers of field-infected peanut. *Peanut Science, 22*, 141–149.

Kritzman, A., Gera, A., Raccah, B., van Lent, J. W., & Peters, D. (2002). The route of *Tomato spotted wilt virus* inside the thrips body in relation to transmission efficiency. *Archives of Virology, 147*(11), 2143–2156. doi: 10.1007/s00705-002-0871-x

Kumm, S., & Moritz, G. (2010). Life-cycle variation, including female production by virgin females in *Frankliniella occidentalis* (Thysanoptera: Thripidae). *Journal of Applied Entomology, 134* 491–497. doi: 10.1111/j.1439-0418.2009.01473.x

Kuwabara, K., Yokoi, N., Ohki, T., & Tsuda, S. (2010). Improved multiplex reverse transcription-polymerase chain reaction to detect and identify five tospovirus species simultaneously. *Journal of General Plant Pathology, 76*(4), 273–277. doi: 10.1007/s10327-010-0246-1

Latham, L. J., & Jones, R. A. C. (1997). Occurrence of tomato spotted wilt tospovirus in native flora, weeds, and horticultural crops. *Australian Journal of Agricultural Research, 48*, 359–369.

Latham, L. J., & Jones, R. A. C. (1998). Selection of resistance breaking strains of Tomato spotted wilt tospovirus. *Annal of Applied Biology, 133*, 385–402.

Leiss, K. A., Choi, Y. H., Abdel-Farid, I. B., Verpoorte, R., & Klinkhamer, P. G. (2009). NMR metabolomics of thrips (*Frankliniella occidentalis*) resistance in Senecio hybrids. *Journal of Chemical Ecology, 35*(2), 219–229. doi: 10.1007/s10886-008-9586-0

Li, R., & Ling, K. S. (2014). Development of reverse transcription loop-mediated isothermal amplification assay for rapid detection of an emerging potyvirus: Tomato necrotic stunt virus. *Journal of Virological Methods, 200*, 35–40. doi: 10.1016/j.jviromet.2014.01.017

Lin, Y. H., Chen, T. C., Hsu, H. T., Liu, F. L., Chu, F. H., Chen, C. C., Lin, Y. Z., & Yeh, S. D. (2005). Serological comparison and molecular characterization for verification of

Calla lily chlorotic spot virus as a new Tospovirus species belonging to Watermelon silver mottle virus serogroup. *Phytopathology, 95*(12), 1482–1488. doi: 10.1094/PHYTO-95-1482

Macharia, I., Backhouse, D., Ateka, E. M., Wu, S. B., Harvey, J., Njahira, M., & Skilton, R. A. (2015). Distribution and genetic diversity of *Tomato spotted wilt virus* following an incursion into Kenya. *Annals of Applied Biology, 166*(3), 520–529. doi: 10.1111/aab.12201

Macharia, I., Backhouse, D., Skilton, R., Ateka, E., Wu, S. B., Njahira, M., Maina, S., & Harvey, J. (2015). Diversity of thrips species and vectors of *Tomato spotted wilt virus* in tomato production systems in Kenya. *Journal of Economic Entomology, 108*(1), 20–28. doi: 10.1093/Jee/Tou010

Maharijaya, A., Vosman, B., Steenhuis-Broers, G., Harpenas, A., Purwito, A., Visser, R. G. F., & Voorrips, R. E. (2011). Screening of pepper accessions for resistance against two thrips species (*Frankliniella occidentalis* and *Thrips parvispinus*). *Euphytica, 177*(3), 401–410. doi: 10.1007/s10681-010-0277-x

Mandal, B., Csinos, A. S., Martinez-Ochoa, N., & Pappu, H. R. (2008). A rapid and efficient inoculation method for Tomato spotted wilt tospovirus. *Journal of Virological Methods, 149*(1), 195–198. doi: 10.1016/j.jviromet.2007.12.007

Maris, P. C., Joosten, N. N., Goldbach, R. W., & Peters, D. (2003a). Restricted spread of *Tomato spotted wilt virus* in thrips-resistant pepper. *Phytopathology, 93*, 1223–1227.

Maris, P. C., Joosten, N. N., Goldbach, R. W., & Peters, D. (2003b). Spread of *Tomato spotted wilt virus* and population development of *Frankliniella occidentalis* in pepper resistant to thrips. *Proceedings of the Section Experimental and Applied Entomology, 14*, 95–101.

Maris, P. C., Joosten, N. N., Goldbach, R. W., & Peters, D. (2004). Tomato spotted wilt virus infection improves host suitability for its vector *Frankliniella occidentalis*. *Phytopathology, 94*, 706–711.

Maris, P. C., Joosten, N. N., Peters, D., & Goldbach, R. W. (2002). Thrips resistance in pepper and its consequences for the acquisition and inoculation of tomato spotted wilt virus by the western flower thrips. *Phytopathology, 93*, 96–101.

Markham R. N., & Zuker, M. (2005). DINAMelt web server for nucleic acid melting prediction. *Nucleic Acids Research, 33*, W577–W581.

Masinde, A. O. A., Kwambai, K. T., & Wambani, N. H. (2011). Evaluation of tomato (*Lycopersicon esculentum* L.) variety tolerance to foliar diseases at Kenya Agricultural

Research Institute Centre-Kitale in North west Kenya. *African Journal of Plant Science 5*, 676–681.

Mason, G., Roggero, P., & Tavella, L. (2003). Detection of *Tomato spotted wilt virus* in its vector *Frankliniella occidentalis* by reverse transcription-polymerase chain reaction. *Journal of Virological Methods, 109*(1), 69–73. doi: 10.1016/s0166-0934(03)00048-x

McDonald, J. R., Bale, J. S., & Walters, K. F. A. (1998). Effect of temperature on developement of the western flower thrips, *Frankiniella occidentalis* (Thysanoptera: Thripidae). *European Journal of Entomology, 95*, 301–306.

McMichael, L., Persley, D., & Thomas, J. (2002). A new tospovirus serogroup IV species infecting capsicum and tomato in Queensland, Australia. *Australasian Plant Pathology, 31*(3), 231–239. doi: 10.1071/ap02016

Melzer, M. J., Tripathi, S., Matsumoto, T., Keith, L., Wieczorek, A., & Gonsalves, D. (2012). Tomato spotted wilt *Plant Disease* (Vol. PD-81). Hawaii: Univerity of Hawaii.

Milne, R. G. (1970). An electron microscope study of Tomato spotted wilt virus in sections of infected cells and in negative stain preparations *Journal of General Virology 6*, 267–276.

Mirnezhad, M., Romero-Gonzalez, R. R., Leiss, K. A., Choi, Y. H., Verpoorte, R., & Klinkhamer, P. G. (2010). Metabolomic analysis of host plant resistance to thrips in wild and cultivated tomatoes. *Phytochemical Analysis, 21*(1), 110–117. doi: 10.1002/pca.1182

Momol, M. T., Olson, S. M., Funderburk, J. E., Stavisky, J., & Marois, J. J. (2004). Integrated management of Tomato spotted wilt on field-grown tomatoes. *Plant Disease, 88*, 882–890.

Moriones, E., Aramburu, J., Riudavets, J., Arno, J., & Lavina, A. (1998). Effect of plant age at time of infection by tomato spotted wilt tospovirus on the yield of field-grown tomato. *European Journal of Plant Pathology, 104*, 295–300.

Moritz, G., Brandt, S., Triapitsyn, S., & Subramanian, S. (2013). *Identification and information tools for pest thrips in East Africa.* Centre for Biological Information Technology (CBIT) Publishing, Queensland, Australia.

Morsello, S. C., Groves, R. L., Nault, B. A., & Kennedy, G. G. (2008). Temperature and precipitation affect seasonal patterns of dispersing tobacco thrips, *Frankliniella fusca*, and onion thrips, *Thrips tabaci* (Thysanoptera: Thripidae) caught on sticky traps. *Environmental Entomology, 37*(1), 79–86. doi: 10.1603/0046-225x(2008)37[79:tapasp]2.0.co;2

Morsello, S. C., & Kennedy, G. G. (2009). Spring temperature and precipitation affect tobacco thrips, *Frankliniella fusca*, population growth and *Tomato spotted wilt virus* spread within patches of the winter annual weed *Stellaria media*. *Entomologia Experimentalis et Applicata, 130*(2), 138–148. doi: 10.1111/j.1570-7458.2008.00801.x

Mound, L. A. (1996). The Thysanoptera vector species of tospoviruses. *Acta Horticulturae, 431*, 298–309.

Mound, L. A. (1997). Biological diversity of thrips. In T. Lewis (Ed.), *Thrips as crop pests* (pp. 197–215). New York: CAB International.

Mound, L. A. (2006). Western flower thrip (*Frankliniella occidentalis*). URL http://www.padil.gov.au/pests-and-diseases/pest/main/136422 [Accessed 18 March 2015].

Mound, L. A. (2007). Onion thrips (*Thrips tabaci*). URL http://www.padil.gov.au/pests-and-diseases/pest/main/136448 [Accessed 18 March 2015].

Mound, L. A., & Azidah, A. A. (2009). Species of the genus *Thrips* (Thysanoptera) from Peninsular Malaysia, with a checklist of recorded Thripidae. *Zootaxa, 2023*, 55–68.

Mound, L. A., & Nickle, D. A. (2009). The old-world genus *Ceratothripoides* (Thysanoptera: Thripidae) with a new genus for related new-world species. *Zootaxa, 2230*, 57–63.

Mound, L. A., Paris, D., & Fisher, N. (2009). *World Thysanoptera; CSIRO.* URL http://anic.ento.csiro.au/thrips [Accessed 12 May 2012)

Mound, L. A., Tree, D. J., & Paris, D. (2014). *OZ THRIPS, Thysanoptera in Australia.* URL http://www.ozthrips.org/terebrantia /thripidae/thripinae/frankliniella-occidentalis/ [Accessed 18 November 2014]

Mumford, R. A., Barker, I., & Wood, K. R. (1996). An improved method for the detection of tospoviruses using the polymerase chain reaction. *Journal of Virological Methods, 57*, 109–115.

Mumford, R. A., Walsh, K., Barker, I., & Boonham, N. (2000). Detection of potato mop top virus and tobacco rattle virus using a multiplex realtime fluorescent reverse-transcription polymerase chain reaction assay. *Phytopathology, 90*, 448–453.

Nagata, T., Almeida, A. C. L., Resende, R. O., & DeÁvila, A. C. (2004). The competence of four thrips species to transmit and replicate four tospoviruses. *Plant Pathology, 53*(2), 136–140. doi: 10.1111/j.0032-0862.2004.00984.x

Nagata, T., Nagata, A. K. I., Lent, J. v., Goldbach, R., & Peters, D. (2002). Factors determining vector competence and specificity for transmission of *Tomato spotted wilt virus*. *Journal of General Virology, 83*, 663–671.

Naidu, R. A., Sherwood, J. L., & Deom, C. M. (2008). Characterization of a vector-non-transmissible isolate of *Tomato spotted wilt virus*. *Plant Pathology, 57*, 190–200.

Naika, S., Jeude, J. v. L. d., Goffau, M. d., Hilmi, M., & Dam, B. v. (2005). *Cultivation of tomato: production, processing and marketing* Wageningen, Netherlands: Agromisa Foundation and CTA, http://www.journeytoforever.org/farm_library/AD17.pdf. [Accessed 19 March 2015].

Nakahara, S. (1997). Annotated list of *Frankliniella* species of the world (Thysanoptera, Thripidae) *Contributions on Entomology, International, 2*(4), 355–384.

Nderitu, J., Mwangi, F., Nyamasyo, G., & Kasina, M. (2010). Utilization of synthetic and botanical insecticides to manage Thrips (Thysan.: Thrip.) on snap beans (Fabaceae) in Kenya. *International Journal of Sustainable Crop Production, 5*(1), 1–4.

Niassy, S., Maniania, N. K., Subramanian, S., Gitonga, L. M., Mburu, D. M., Masiga, D., & Ekesi, S. (2012). Selection of promising fungal biological control agent of the western flower thrips *Frankliniella occidentalis* (Pergande). *Letters in Applied Microbiology 54*(6), 487–493. doi: 10.1111/j.1472765X.2012.03241.x

Northfield, T. D., Paini, D. R., Funderburk, J. E., & Reitz, S. R. (2008). Annual cycles of *Frankliniella* spp. (Thysanoptera: Thripidae) thrips abundance on North Florida uncultivated reproductive hosts: Predicting possible sources of pest outbreaks. *Annals of the Entomological Society of America, 101*(4), 769–778. doi: 10.1603/0013-8746(2008)101[769:acofst]2.0.co;2

Nyasani, J. O., Meyho¨fer, R., Subramanian, S., & Poehling, H.-M. (2010). Thrips species composition and abundance on French beans, associated crops and weed species in Kenya. *Journal of Insect Science, 10*, 166.

Nyasani, J. O., Meyhöfer, R., Subramanian, S., & Poehling, H. M. (2012). Effect of intercrops on thrips species composition and population abundance on French beans in Kenya. *Entomologia Experimentalis et Applicata, 142*(3), 236–246. doi: 10.1111/j.1570-7458.2011.01217.x

Nyasani, J. O., Meyhöfer, R., Subramanian, S., & Poehling, H. M. (2013). Feeding and oviposition preference of *Frankliniella occidentalis* for crops and weeds in Kenyan French bean fields. *Journal of Applied Entomology, 137*(3), 204–213. doi: 10.1111/j.1439-0418.2012.01723.x

Nyasani, J. O., Subramanian, S., Poehling, H.-M., Maniania, N. K., Ekesi, S., & Meyhöfer, R. (2015). Optimizing western flower thrips management on French beans by combined use of beneficials and Imidacloprid. *Insects, 6*, 279–296. doi: 10.3390/insects6010279

Ochoa Martinez, D. L., Zavaleta-Mejıa, E., Mora-Aguilera, G., & M., J. N. R. (1999). Implications of weed composition and thrips species for the epidemiology of tomato spotted wilt in chrysanthemum (*Dendranthema grandiflora*). *Plant Pathology, 48*, 707–717.

Ohnishi, J., Katsuzaki, H., & Tsuda, S. (2006). *Frankiniela cephalica,* a new vector for *Tomato spotted wilt virus*. *Plant Disease 80*, 685.

Okazaki, S., Okuda, M., Komi, K., Yamasaki, S., Okuda, S., Sakurai, T., & Iwanami, T. (2011). The effect of virus titre on acquisition efficiency of *Tomato spotted wilt virus* by *Frankliniella occidentalis* and the effect of temperature on detectable period of the virus in dead bodies. *Australasian Plant Pathology, 40*(2), 120–125. doi: 10.1007/s13313-010-0020-z

Okazaki, S., Okuda, M., Komi, K., Yoshimatsu, H., & Iwanami, T. (2007). Overwintering viruliferous *Frankliniella occidentalis* (Thysanoptera : Thripidae) as an infection source of *Tomato spotted wilt virus* in green pepper fields. *Plant Disease, 91*(7), 842–846. doi: 10.1094/pdis-91-7-0842

Okazaki, S., Okuda, M., & Sakurai, T. (2009). Evaluation of weed species as *Tomato spotted wilt virus* (Bunyaviridae: Tospovirus) acquisition sources for *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). *Japanese Journal of Applied Entomology and Zoology, 53*(4), 181–184. doi: 10.1303/jjaez.2009.181

Olatinwo, R. O., Prabha, T., Paz, J. O., Riley, D. G., & Hoogenboom, G. (2011). The Weather Research and Forecasting (WRF) model: Application in prediction of TSWV-vectors populations. *Journal of Applied Entomology, 135*(1-2), 81–90. doi: 10.1111/j.1439-0418.2010.01539.x

Olembo, S. A. H. (2001). *Inter-African quarantine and phytosanitary regulatory principles for viruses in plant introductions.* Paper presented at the Plant Virology in Sub-Saharan Africa, International Institute of Torical Agriculture, Ibadan, Nigeria.

Otipa, M. J., Kimenju, J. W., Mureithi, J. G., & Kyalo, G. (2009). Potential of rotation crops in managing root knot (*Meloidogyne* spp.) nematodes in tomato. *African Journal of Horticultural Science 2*, 111–123.

Packer, L., Gibbs, J., Sheffield, C., & Hanner, R. (2009). DNA barcoding and the mediocrity of morphology. *Molecular Ecology Resources, 9*(S1), 42–50. doi: 10.1111/j.1755-0998.2009.02631.x

Panthee, D. R., & Foolad, M. R. (2011). A re-examination of molecular markers for use in marker-assisted breeding in tomato. *Euphytica, 184*(2), 165–179. doi: 10.1007/s10681-011-0544-5

Papadaki, M., Harizanova, V., & Dagnon, S. (2008). Influence of feeding of *Frankliniella occidentalis* Pergande (Thysanoptera:Thripidae) on the polyphenolic complex in the leaves. *Bulgarian Journal of Agricultural Science, 14*(4), 405–409.

Pappu, H. R. (2008). *Tomato spotted wilt virus* (Bunyaviridae) In B. W. J. Mahy & M. H. V. van Regenmortel (Eds.), *Encyclopedia of Virology* (Vol. 5, pp. 133–138). Oxford, UK: Elservier.

Pappu, H. R., Jones, R. A. C., & Jain, R. K. (2009). Global status of tospovirus epidemics in diverse cropping systems: Successes achieved and challenges ahead. *Virus Research, 141*(2), 219–236. doi: 10.1016/j.virusres.2009.01.009

Parrella, G., Gognalons, P., Gebre-Selassiè, Vovlas, K., & Marchoux, C. G. (2003). An update of the host range of *Tomato spotted wilt virus*. *Journal of Plant Pathology, 85*(4), 227–264.

Peiro, A., Canizares, M. C., Rubio, L., Lopez, C., Moriones, E., Aramburu, J., & Sanchez-Navarro, J. (2014). The movement protein (NSm) of *Tomato spotted wilt virus* is the avirulence determinant in the tomato *Sw-5* gene-based resistance. *Molecular Plant Pathology 15*(8), 802–813. doi: 10.1111/mpp.12142

Persley, D. M., Thomas, J. E., & Sharman, M. (2006). Tospoviruses—an Australian perspective. *Australasian Plant Pathology, 35*, 161–180. doi: 10.1071/ap06015

Premachandra, D. W. T. S., Borgemeister, C., Sétamou, M., Achilles, T., & Poehling, H.-M. (2005). Spatio-temporal distribution of *Ceratothripoides claratris* (Thysanoptera: Thripidae) on tomatoes in Thailand. *Environmental Entomology, 34*(4), 883–890. doi: 10.1603/0046-225x-34.4.883

Rahman, T., Spafford, H., & Broughton, S. (2011). Single versus multiple releases of predatory mites combined with spinosad for the management of western flower thrips in strawberry. *Crop Protection, 30*(4), 468–475. doi: 10.1016/j.cropro.2010.11.027

Ramkat, R. C., Wangai, A. W., Ouma, J. P., Rapando, P. N., & Legut, D. K. (2006). Effect of mechanical inoculation of *Tomato spotted wilt virus* tospovirus disease on disease severity and yield of greenhouse raise tomatos. *Asia Journal of Plant Sciences, 5*(4), 607–612.

Ramkat, R. C., Wangai, A. W., Ouma, J. P., Rapando, P. N., & Lelgut, D. K. (2008). Cropping system influences *Tomato spotted wilt virus* disease development, thrips

population and yield of tomato (*Lycopersicon esculentum*). *Annals of Applied Biology, 153*(3), 373–380. doi: 10.1111/j.1744-7348.2008.00268.x

Rice, D., J., German, T. L., Mau, R. F., & Fujimoto, F. M. (1990). Dot blot detection of *Tomato spitted wilt virus* RNA in plant and thrips tissues cDNA clones. *Plant Disease, 74*, 274–276.

Reitz, S. R., 2009. Biology and ecology of the Western flower thrips (Thysanoptera: Thripidae): The making of a pest. Florida Entomologist, 92(1):7-13.

Riley, D. G., Joseph, S. V., Kelley, W. T., Olson, S., & Scott, J. (2011a). Host plant resistance to *Tomato spotted wilt virus* (Bunyaviridae: Tospovirus) in tomato. *Hortscience, 46*(12), 1626–1633.

Riley, D. G., Joseph, S. V., Srinivasan, R., & Diffie, S. (2011b). Thrips vectors of tospoviruses. *Journal of Integrated Pest Management, 2*(1), 1–10. doi: 10.1603/ipm10020

Riley, D. G., & Pappu, H. R. (2004). Tactics for management of thrips (Thysanoptera: Thripidae) and *Tomato spotted wilt virus* in tomato. *Journal of Economic Entomology, 97*(5), 1648–1658. doi: 10.1603/0022-0493-97.5.1648

Roberts, C. A., Dietzgen, R. G., Heelan, L. A., & Maclean, D. J. (2000). Real-time RT-PCR fluorescent detection of *Tomato spotted wilt virus*. *Journal of Virological Methods 88* 1–8.

Rosello, S., Diez, M. J., & Nuez, F. (1996). Viral diseases causing the greatest economic losses to the tomato crop. I. The *Tomato spotted wilt virus* - a review. *Scientia Horticulturae, 67*, 117–150.

Rosello, S., Dıez, M. J., & Nuez, F. (1998). Genetics of tomato spotted wilt virus resistance coming from *Lycopersicon peruvianum*. *European Journal of Plant Pathology, 104*, 499–509.

Rotenberg, D., Krishna Kumar, N. K., Ullman, D. E., Montero-Astua, M., Willis, D. K., German, T. L., & Whitfield, A. E. (2009). Variation in *Tomato spotted wilt virus* titer in *Frankliniella occidentalis* and its association with frequency of transmission. *Phytopathology, 99*(4), 404–410. doi: 10.1094/PHYTO-99-4-0404

Rugman-Jones, P. F., Hoddle, M. S., & Stouthamer, R. (2010). Nuclear-mitochondrial barcoding exposes the global pest western flower thrips (Thysanoptera: Thripidae) as two sympatric cryptic species in its native California. *Journal of Economic Entomology, 103*(3), 877–886. doi: 10.1603/ec09300

Saidi, M., & Warade, S. D. (2008). Tomato breeding for resistance to Tomato spotted wilt virus (TSWV): An overview of conventional and molecular approaches. *Czech Journal of Genetics and Plant Breeding, 44*(3), 83–92.

Sakimura, K. (1969). A comment on the color forms of *Frankliniella schultzei* (Thysanoptera: Thripidae) in relation to transmission of the *Tomato spotted wilt virus*. *Pacific Insects, 11* 761–762.

Sakurai, T. (2004). Transmission of *Tomato spotted wilt virus* by the dark form of *Frankliniella schultzei* (Thysanoptera: Thripidae) originating in tomato fields in Paraguay. *Applied Entomology and Zoology, 39* (1), 189–194.

Samuel, G., Bald, J. G., & Pittman, H. A. (1930). Investigations on 'spotted wilt' of tomatoes. *Australian Council of Science and Industrial Research Bulletin* 44, pp. 64.

Scholthof, K. B., Adkins, S., Czosnek, H., Palukaitis, P., Jacquot, E., Hohn, T., Hohn, B., Saunders, K., Candresse, T., Ahlquist, P., Hemenway, C., & Foster, G. D. (2011). Top 10 plant viruses in molecular plant pathology. *Molecular Plant Pathology, 12*(9), 938–954. doi: 10.1111/j.1364-3703.2011.00752.x

Scott Brown, A. S., Simmonds, M. S. J., & Blaney, W. M. (2002). Relationship between nutritional composition of plant species and infestation levels of thrips. *Journal of Chemical Ecology, 28*(12), 2399–2409.

Seepiban, C., Gajanandana, O., Attathom, T., & Attathom, S. (2011). Tomato necrotic ringspot virus, a new tospovirus isolated in Thailand. *Archives of Virology, 156*(2), 263–274. doi: 10.1007/s00705-010-0856-0

Sevik, M. A., & Arli-Sokmen, M. (2012). Estimation of the effect of *Tomato spotted wilt virus* (TSWV) infection on some yield components of tomato. *Phytoparasitica, 40*(1), 87–93. doi: 10.1007/s12600-011-0192-2

Sharman, M., & Persley, D. M. (2006). Field isolates of Tomato spotted wilt virus overcoming resistance in capsicum in Australia. *Australasian Plant Pathology, 35*(2), 123. doi: 10.1071/ap06014

Sherwood, J. L., Sanborn, M. R., Keyser, G. C., & Myers, L. D. (1989). Use of monoclonal antibodies in detection of tomato spotted wilt virus. *Phytopathology, 79*, 61–64.

Shi, A., & Vierling, R. (2011). Identification of molecular markers for *Sw-5* gene of tomato spotted wilt virus resistance. *American Journal of Biotechnology and Molecular Sciences, 1*(1), 8–16. doi: 10.5251/ajbms.2011.1.1.8.16

Shimomoto, Y., Kobayashi, K., & Okuda, M. (2014). Identification and characterization of Lisianthus necrotic ringspot virus, a novel distinct tospovirus species causing necrotic

disease of lisianthus (*Eustoma grandiflorum*). *Journal of General Plant Pathology, 80*(2), 169–175. doi: 10.1007/s10327-014-0503-9

Shrestha, A., Srinivasan, R., Riley, D. G., & Culbreath, A. K. (2012). Direct and indirect effects of a thrips-transmitted Tospovirus on the preference and fitness of its vector, *Frankliniella fusca*. *Entomologia Experimentalis et Applicata, 145*(3), 260–271. doi: 10.1111/eea.12011

Silva, M. S., Martins, C. R. F., Bezerra, I. C., Nagata, T., de Ávila, A. C., & Resende, R. O. (2001). Sequence diversity of NSM movement protein of tospoviruses. *Archives of Virology, 146*, 1267–1281.

Sin, S. H., McNulty, B. C., Kennedy, G. G., & Moyer, J. W. (2005). Viral genetic determinants for thrips transmission of Tomato spotted wilt virus. *Proceedings of the National Academy of Sciences of the United States of America, 102*(14), 5168–5173. doi: 10.1073/pnas.0407354102

Sivparsad, B., & Gubba, A. (2011). Evaluation of tomato (*Lycopersicon esculentum* Mill.) plants with natural and transgenic resistance against *Tomato spotted wilt virus* (TSWV) isolates occurring in the Republic of South Africa (RSA). *African Journal of Agricultural Research, 6*(13), 3013–3020.

Sivparsad, B. J., & Gubba, A. (2008). Isolation and molecular characterization of *Tomato spotted wilt virus* (TSWV) isolates occurring in South Africa. *African Journal of Agricultural Research 3*(6), 428–434.

Soler, S., Cebolla-Cornejo, J., & Nuez, F. (2003). Control of diseases induced by tospoviruses in tomato an update of genetic approach. *Phytopathologia Mediterranea, 42*, 207–219.

Spassova, M. I., Prins, T. W., Folkertsma, R. T., Klein-Lankhorst, R. M., Hille, J., Goldbach, R. W., & Prins, M. (2001). The tomato gene *Sw-5* is a member of the coiled coil, nucleotide binding, leucine-rich repeat class of plant resistance genes and confers resistance to TSWV in tobacco. *Molecular Breeding 7*, 151–161.

Srinivasan, R. (2010). *Safer tomato production methods: A guide for soil fertility and pest management*. The World Vegetable Center, Shnhua, Taiwan: AVRDC Publication, No. 10-740 97p.

Srinivasan, R., Riley, D., Diffie, S., Shrestha, A., & Culbreath, A. (2014). Winter weeds as inoculum sources of *Tomato spotted wilt virus* and as reservoirs for its vector, *Frankliniella fusca* (Thysanoptera: Thripidae) in farmscapes of Georgia. *Environmental Entomology, 43*(2), 410–420. doi: 10.1603/EN13288

Srinivasan, R., Sundaraj, S., Pappu, H. R., Diffie, S., Riley, D. G., & Gitaitis, R. D. (2012). Transmission of *Iris yellow spot virus* by *Frankliniella fusca* and *Thrips tabaci* (Thysanoptera: Thripidae). *Journal of Economic Entomology, 105*(1), 40–47. doi: 10.1603/ec11094

Ssekyewa, C. (2006). *Incidence, distribution and characteristics of major tomato leaf curl and mosaic virus diseases in Uganda.* Applied Biological Sciences PhD Thesis, Ghent University, Ghent, Belgium.

Steenken, N., & Halawel, N. (2011). Host prevalence study for *Ceratothripiodes claratris* (Shumsher) (Thysanoptera: Thripidae) and CaCV (Genus *Tospovirus*: family Bunyaviridae) in Bangkok, Thailand. *Journal of Entomology, 8*(2), 198–208.

Stevens, M. R., Lamb, E. M., & Rhoads, D. D. (1995). Mapping the *Sw-5* locus for tomato spotted wilt virus resistance in tomatoes using RAPD and RFLP analyses. *Theoretical and Applied Genetics, 90*, 451–456.

Stevens, M. R., Scott, J. W., Geary, B. D., Cho, J. J., Gordillo, L. F., Persley, D. M., Memmott, F. D., & Stevens, J. J. (2006). *Current status of resistance to tospoviruses in tomato* Paper presented at the Tomato Breeders Roundtable and Tomato QualityWorkshop, Tampa, Forida, USA.

Stevens, M. R., Scott, S. J., & Gergerich, R. C. (1994). Evaluation of seven *Lycopersicon* species for resistance to tomato spotted wilt virus (TSWV). *Euphytica 80*, 79–84.

Strange, M., Schrader, W. L., & Hartz, T. K. (2000). *Fresh market tomato production in California*. Vegetable research and information center; vegetable production series, (8017). University of California, http://anrcatalog.ucdavis.edu/pdf/8017.pdf.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution, 28*(10), 2731–2739. doi: 10.1093/molbev/msr121

Tentchev, D., Verdin, E., Marchal, C., Jacquet, M., Aguilar, J. M., & Moury, B. (2011). Evolution and structure of Tomato spotted wilt virus populations: evidence of extensive reassortment and insights into emergence processes. *Journal of General Virology, 92*(Pt 4), 961–973. doi: 10.1099/vir.0.029082-0

Thomas-Carroll, L., & Jones, R. A. C. (2003). Selection, biological properties and fitness of resistance-breaking strains of *Tomato spotted wilt virus* in pepper. *Annals of Applied Biology, 142*, 235–243.

Thompson, G. J., & van Zijl, J. J. B. (1996). Control of tomato spotted wilt virus in tomatoes in South Africa. *Acta Horticulturae, 431*, 379–384.

Timm, A. E., Stiller, M., & Frey, J. E. (2008). A molecular identification key for economically important thrips species (Thysanoptera: Thripidae) in southern Africa. *African Entomology, 16*(1), 68–75. doi: 10.4001/1021-3589-16.1.68

Timmerman-Vaughan, G. M., Lister, R., Cooper, R., & Tang, J. (2014). Phylogenetic analysis of New Zealand *Tomato spotted wilt virus* isolates suggests likely incursion history scenarios and mechanisms for population evolution. *Archives of Virology, 159*, 993–1003. doi: 10.1007/s00705-013-1909-y

Toda, S., & Morishita, M. (2009). Identification of three point mutations on the sodium channel gene in Pyrethroid-resistant *Thrips tabaci* (Thysanoptera: Thripidae). *Journal of Economic Entomology, 102*(6), 2296–2300. doi: 10.1603/029.102.0635

Toda, S., & Murai, T. (2007). Phylogenetic analysis based on mitochondrial COI gene sequences in *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) in relation to reproductive forms and geographical distribution. *Applied Entomology and Zoology, 42*(2), 309–316. doi: http://dx.doi.org/10.1303/aez.2007.309

Torres, R., Larenas, J., Fribourg, C., & Romero, J. (2012). Pepper necrotic spot virus, a new tospovirus infecting solanaceous crops in Peru. *Archives of Virology, 157*(4), 609–615. doi: 10.1007/s00705-011-1217-3

Tree, D. J. (2010). *Tomato thrips* (*Frankliniella schultzei*). URL http://www.padil.gov.au/pests-and-diseases/pest/main/139898 [Accessed 18 March 2015].

Tsompana, M., Abad, J., Urugganan, M. P., & Moyer, J. W. (2005). The molecular population genetics of the *Tomato spotted wilt virus* (TSWV) genome. *Molecular Ecology 14*, 53–66. doi: 10.1111/j.1365-294X.2004.02392.x

Tsuda, S., Fujisawa, I., Ohnishi, J., Hosokawa, D., & Tomaru, K. (1996). Localization of tomato spotted wilt tospovirus in larvae and pupae of the insect vector *Thrips setosus*. *Phytopathology, 86*, 1199–1203.

Uga, H., & Tsuda, S. (2005). A one-step reverse transcription-polymerase chain reaction system for the simultaneous detection and identification of multiple tospovirus infections. *Phytopathology, 95*(2), 166–171. doi: 10.1094/PHYTO-95-0166

Ullman, D. E., Westcot, D. M., Chenault, K. D., Sherwood, J. L., German, T. L., & Bandla, M. D. (1995). Compartmentalization, intercellular transport, and autophagy of tomato spotted wilt tospovirus protein in infected thrips cells. *Phytopathology, 85*, 644–654.

Ullman, D. E., Westcot, D. M., Hunter, W. B., & Mau, R. F. L. (1989). Internal anatomy and morphology of *Frankliniella occidentalis* (Pergande) (Thysanoptera : Thripidae) with special reference to interactions between thrips and Tomato spotted wilt virus. *International Journal of Insect Morphology and Embryology, 18*, 289–310.

van de Wetering, F. (1999). *Effects of thrips feeding on tospovirus transmission in chrysanthemum.* PhD. Thesis, Wageningen University, Wageningen, the Netherlands.

Varela, A. M., Seif, A., & Löhr, B. (2003). *A guide to IPM in tomato production in Eastern and Southern Africa* Naorobi, Kenya: ICIPE Science Press.

VSN International. (2014). GenStat for Windows 17th Edition. VSN International, Hemel Hempstead, UK, from https://www.vsni.co.uk/

Waiganjo, M. M., Gitonga, L. M., & Mueke, J. M. (2008). Effects of weather on thrips population dynamics and its implications on the thrips pest management. *Africa Journal of Horticultural Science 1*, 82–90.

Waiganjo, M. M., Wabule, N. M., Nyongesa, D., Kibaki, J. M., Onyango, I., Wepukhulu, S. B., & Muthoka, N. M. (2006). *Tomato production in Kirinyaga district in Kenya, a baseline survey report* (pp. 1–43). KARI/IPM-CRSP Collaborative Project: Kenya Agricultural Research Institute.

Walsh, K., Boonham, N., Barker, I., & Collins, D. W. (2005). Development of a sequence-specific real-time PCR to the melon thrips *Thrips palmi* (Thysan., Thripidae). *Journal of Applied Entomology, 129*(5), 272–279. doi: 10.1111/j.1439-0418.2005.00960.x

Wang, C.-L., Lin, F.-C., Chiu, Y.-C., & Shih, H.-T. (2010). Species of *Frankliniella* Trybom (Thysanoptera: Thripidae) from the Asian-Pacific Area. *Zoological Studies, 49*(6), 824–838

Wangai, A. W., Mandal, B., Pappu, H. R., & Kilonzo, S. (2001). Outbreak of *Tomato spotted wilt virus* in tomato in Kenya. *Plant Disease 85*(10), 1123.

Westmore, G. C., Poke, F. S., Allen, G. R., & Wilson, C. R. (2013). Genetic and host-associated differentiation within *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) and its links to tomato spotted wilt virus-vector competence. *Heredity, 111*, 210–215.

Whitfield, A. E., Campbell, L. R., Sherwood, J. L., & Ullman, D. E. (2003). Tissue blot immunoassay for detection of Tomato spotted wilt virus in Ranunculus asiaticus and other ornamentals. *Plant Disease, 87*, 618–622.

Whitfield, A. E., Kumar, N. K., Rotenberg, D., Ullman, D. E., Wyman, E. A., Zietlow, C., Willis, D. K., & German, T. L. (2008). A soluble form of the Tomato spotted wilt virus (TSWV) glycoprotein G(N) (G(N)-S) inhibits transmission of TSWV by

Frankliniella occidentalis. *Phytopathology, 98*(1), 45–50. doi: 10.1094/PHYTO-98-1-0045

Whitfield, A. E., Ullman, D. E., & German, T. L. (2005). Tospovirus-thrips interactions. *Annual Review of Phytopathology, 43*, 459–489. doi: 10.1146/annurev.phyto.43.040204.140017

Wijkamp, I., Almarza, N., Goldbach, R., & Peters, D. (1995). Distinct levels of specificity in thrips transmission of tospoviruses. *Phytopathology 85*, 1069–1074.

Wijkamp, I., van Lent, J., Kormelink, R., Goldbach, R., & Peters, D. (1993). Multiplication of *Tomato spotted wilt virus* in its insect vector, *Frankliniella occidentalis*. *Journal of General Virology, 74*, 341–349.

Wilson, C. R. (1998). Incidence of weed reservoirs and vectors of tomato spotted wilt tospovirus on southern Tasmanian lettuce farms. *Plant Pathology, 47*(2), 171–176. doi: 10.1046/j.1365-3059.1998.00227.x

Wilson, C. R. (2001). Resistance to infection and translocation of *Tomato spotted wilt virus* in potatoes. *Plant Pathology, 50*(3), 402–410. doi: 10.1046/j.1365-3059.2001.00562.x

Wu, J., Yu, C., Yang, C., & Zhou, X. (2009). Monoclonal antibodies against the recombinant nucleocapsid protein of *Tomato spotted wilt virus* and its application in virus detection. *Journal of Phytopathology, 157*(6), 344–349. doi: 10.1111/j.1439-0434.2008.01498.x

Wu, S.-B., Wirthensohn, M. G., Michelle, G., Hunt, P., Gibson, J. P., & Sedgley, M. (2008). High resolution melting analysis of almond SNPs derived from ESTs. *Theoretical and Applied Genetics, 118*(1), 1–14.

Xu, Y., Lou, S. G., Li, Q. F., Hu, Q., Sun, L. P., Li, Z. Y., Li, Y. Z., & Liu, Y. T. (2014). Molecular characterization of the hippeastrum chlorotic ringspot virus L segment and its protein. *Archives of Virology, 159*(10), 2805–2807. doi: 10.1007/s00705-014-2100-9

Yang, W., Bai, X., Kabelka, E., Eaton, C., Kamoun, S., van der Knaap, E., & Franscis, D. (2004). Discovery of single nucleotide polymorphisms in *Lycopersicon esculentum* by computer aided analysis of expressed sequence tags. *Molecular Breeding, 14*, 21–34.

Zhang, G. F., Meng, X. Q., Min, L., Qiao, W. N., & Wan, F. H. (2012). Rapid diagnosis of the invasive species, *Frankliniella occidentalis* (Pergande): a species-specific COI marker. *Journal of Applied Entomology, 136*(6), 410–420. doi: 10.1111/j.1439-0418.2011.01661.x

Zhao, G., Liu, W., Brown, J. M., & Knowles, O. (1995). Insecticide resistance in field and laboratory strains of western flower thrips (Thysanoptera; Thripidae). *Journal of Economic Entomology, 88*, 1164–1170.

Zhou, J., Kantartzi, S. K., Wen, R. H., Newman, M., Hajimorad, M. R., Rupe, J. C., & Tzanetakis, I. E. (2011). Molecular characterization of a new *Tospovirus* infecting soybean. *Virus Genes, 43*(2), 289–295. doi: 10.1007/s11262-011-0621-9

Appendices

Appendix 1. Supplementary data for Chapter 3

a) Questionnaire

Survey on occurrence of *Tomato spotted wilt virus*, thrips vectors and weed species in tomato production systems in Kenya.

Section 1: Basic information on the location

Field Number……

Question	Question	Answer
Q1	Province	
Q2	District	
Q3	Division	
Q4	Location	
Q5	GPS coordinates, Decimal Degrees (X)	
Q6	GPS coordinates, Decimal Degrees (Y)	
Q8	Altitude (m)	
Q9	Cropping season	
Q10	Farm size	
Q11	Age of the crop	
Q12	Watering system	
Q13	Name of the household head	
Q14	Name of sampler	
Q15	Sampling date	

Section 2: Information on tomato production

2.1 What variety of tomato do you usually grow?:-

a) .....
b) .....
c) .....
d) .....
e) .....

2.2 Where do you source your seeds? Own seeds ( ), Purchase new seeds ( ) Other ( )

2.3 What are the major constraints in tomato production. Rank them from the most important

1. .....
2. .....
3. .....
4. .....
5. .....

2.4 What pests and diseases do you encounter during tomato production

1. .....
2. .....
3. .....
4. .....
5. .....

2.5 Do you encounter thrips in your farm? Yes ( ), No ( )

2.5b How do you control them?

1. .....
2. .....
3. .....

2.6 What virus diseases have you observed in you farm? (List virus and the local name)

a) .....
b) .....
c) .....
d) .....
e) .....

2.7 Show picture of TSWV, do you observed this type of disease in you farm?

Yes ( ), No ( )

2.8 What month (s) of the year is the disease severe?

2.9 How do you compare infection this year with previous year?

Low ( ), Medium ( ), High ( )

3.0 Do you know any varieties that are resiststant to this disease? Yes ( ), No ( ),

List any such variety:

1. .....
2. .....
3. .....
4. .....
5. .....

Information on plant samples collected

Sample Number	Describe symptoms (CR, Y, S, M, P)	Part of plant	Severity	Test	Sample collected

Comment:

Photograph taken: …….. Describe photograph taken ……

Information on thrips sample

Thrips sample No.	Host plant	Adult/Larva	Population	Sample collected

Section 3: Weeds, growth stage, population, vector presence, TSWV symptoms

(Note: Weeds to be added to the list as they are observed in the production areas)

Botanical name	**Common name**	**Growth stage:**	**Weed population (per quadrant)**					**Sampling site**
			Q1	**Q2**	**Q3**	**Q4**	**Q5**	
Bidens pilosa L	Farmer's friend, black jack							
Tagetes minuta	Mexican marigolds							
Galinsoga parviflora	Gallant soldier							
Datura stramonium	Thorn apple							
Amaranthus hybridus	Pigweed							
Commelina benghalensis	Wandering jew							
Oxygonum sinuatum	Double thorn							
Sonchus oleraceus	Sow thistle							
Solanum nigrum	Black nightshade							
Brassica napus	Rape Weed							
Cleome gynandra	Spider weed							
Malva spp	Mallow							
Chenopodium album	Lamb quarter							
Oxalis latifolia	Oxalis							
Oxalis conculata	Oxalis							

Table A1. A list of tomato spotted wilt virus (TSWV) sequences submitted to GenBank

Isolate	GenBank Number	Host/ Source
NS56	KF963116	Tomato
NS64	KF963117	Tomato
NS175	KF963118	Tomato
NS176	KF963119	Tomato
NS183	KF963120	Tomato
NS184	KF963121	Tomato
T3	KF963122	Thrips
T16	KF963123	Thrips
G1	KF963124	*Datura stramonium**
NS14	KF963125	Tomato
NS22	KF963126	Tomato
NS196	KF963127	Tomato
LO2	KF964052	Tomato
G2	KF964053	*Datura stramonium**
NS59	KF964054	Tomato
NS112	KF964055	Tomato
NS121	KF964056	Tomato
NS109	KF964057	Tomato
NS130	KF964058	Tomato
NS149	KF964059	Tomato
NS159	KF964060	Tomato
NS185	KF964061	Tomato
NS194	KF964062	Tomato

LO-Samples from Loitokitok

NS- Sample from Nakuru area

T- Samples extracted from thrips

*Ex-tomato inoculated on *Datura stramonium* in the greenhouse (Labelled G)

Appendix 2. Supplementary data and figures for Chapter 4

Table A2. A list of thrips species sequences submitted to the GenBank

Isolate*	GenBank	Species
LO2	KF778754	*Ceratothripoides brunneus*
LO4	KF778755	*Ceratothripoides brunneus*
LO10	KF778756	*Ceratothripoides brunneus*
LO12	KF778757	*Ceratothripoides brunneus*
LO21	KF778758	*Ceratothripoides brunneus*
LO22	KF778759	*Ceratothripoides brunneus*
LO24	KF778760	*Ceratothripoides brunneus*
LO29	KF778761	*Ceratothripoides brunneus*
LO32	KF778762	*Ceratothripoides brunneus*
LO48	KF778763	*Ceratothripoides brunneus*
K77	KJ939412	*Ceratothripoides brunneus*
N74	KJ939413	*Ceratothripoides brunneus*
BT19	KJ939414	*Ceratothripoides brunneus*
BT96	KJ939415	*Ceratothripoides brunneus*
LO3	KF765376	*Frankliniella occidentalis*
LO11	KF765377	*Frankliniella occidentalis*
LO13	KF765378	*Frankliniella occidentalis*
LO14	KF765379	*Frankliniella occidentalis*
LO15	KF765380	*Frankliniella occidentalis*
LO23	KF765381	*Frankliniella occidentalis*
LO24	KF765382	*Frankliniella occidentalis*
LO30	KF765383	*Frankliniella occidentalis*
LO53	KF765384	*Frankliniella occidentalis*
N10	KJ939422	*Frankliniella occidentalis*
N114	KJ939423	*Frankliniella occidentalis*
K22	KJ939424	*Frankliniella occidentalis*
K52	KJ939425	*Frankliniella occidentalis*
LO35	KF778777	*Frankliniella schultzei*
LO58	KF778780	*Frankliniella schultzei*
LO60	KF778782	*Frankliniella schultzei*
N106	KJ939419	*Frankliniella schultzei*
K5	KJ939420	*Frankliniella schultzei*
K17	KJ939421	*Frankliniella schultzei*
LO34	KF778776	*Frankliniella tritici*
LO52	KF778771	*Haplothrips globiceps*
LO6	KF778769	*Haplothrips leucanthemi*
LO9	KF778770	*Haplothrips tritici*
LO30	KF778774	*Megalurothrips distalis*
LO7	KF778772	*Scirtothrips dorsalis*
LO25	KF778773	*Scirtothrips dorsalis*
LO59	KF778781	*Scirtothrips* sp *IM2013*
LO33	KF778775	*Thrips sentipennis*

Table A2. Continued

Isolate*	GenBank	Species
LO37	KF778778	*Thrips sentipennis*
LO51	KF778779	*Thrips sentipennis*
N7	KF640664	*Thrips tabaci*
N30	KF640665	*Thrips tabaci*
N60	KF640666	*Thrips tabaci*
N71	KF640667	*Thrips tabaci*
N77	KF640668	*Thrips tabaci*
N78	KF640669	*Thrips tabaci*
N84	KF640670	*Thrips tabaci*
N88	KF640671	*Thrips tabaci*
N91	KF640672	*Thrips tabaci*
N98	KF640673	*Thrips tabaci*
N100	KF640674	*Thrips tabaci*
N101	KF640675	*Thrips tabaci*
N109	KF640676	*Thrips tabaci*
K14	KF640677	*Thrips tabaci*
M25	KF640678	*Thrips tabaci*
K27	KF640679	*Thrips tabaci*
M43	KF640680	*Thrips tabaci*
K44	KF640681	*Thrips tabaci*
LO36	KF778764	*Thrips tabaci*
LO39	KF778765	*Thrips tabaci*
LO42	KF778766	*Thrips tabaci*
LO55	KF778767	*Thrips tabaci*
LO55B	KF778768	*Thrips tabaci*
N2	KJ939416	*Thrips tabaci*
N22	KJ939417	*Thrips tabaci*
ILRI 3	KJ939418	*Thrips tabaci*

* N- samples from Nakuru, LO- samples from Loitokitok, BT- samples from Bungoma and K & M- samples from Kirinyaga

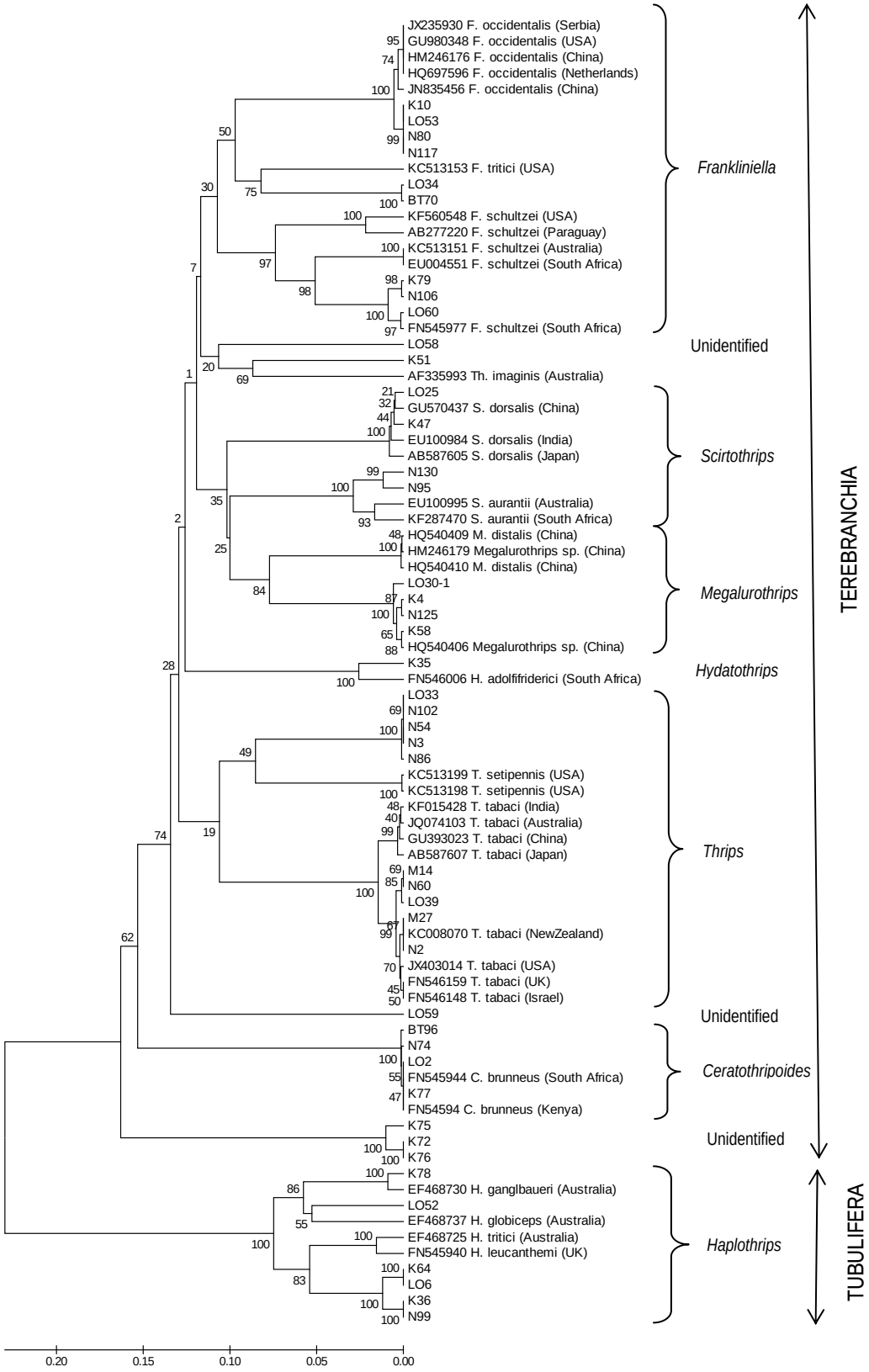

Fig. A1. UPGMA tree of cytochrome oxidase 1 sequences of thrips collected from tomato production areas in Kenya, and databased sequences. Kenyan samples are labeled N (Nakuru), K and M (Kirinyaga), LO (Loitokitok) and BT (Bungoma). The Kimura 2-parameter distance model was used. Bootstrap values are from 1000 replicates.

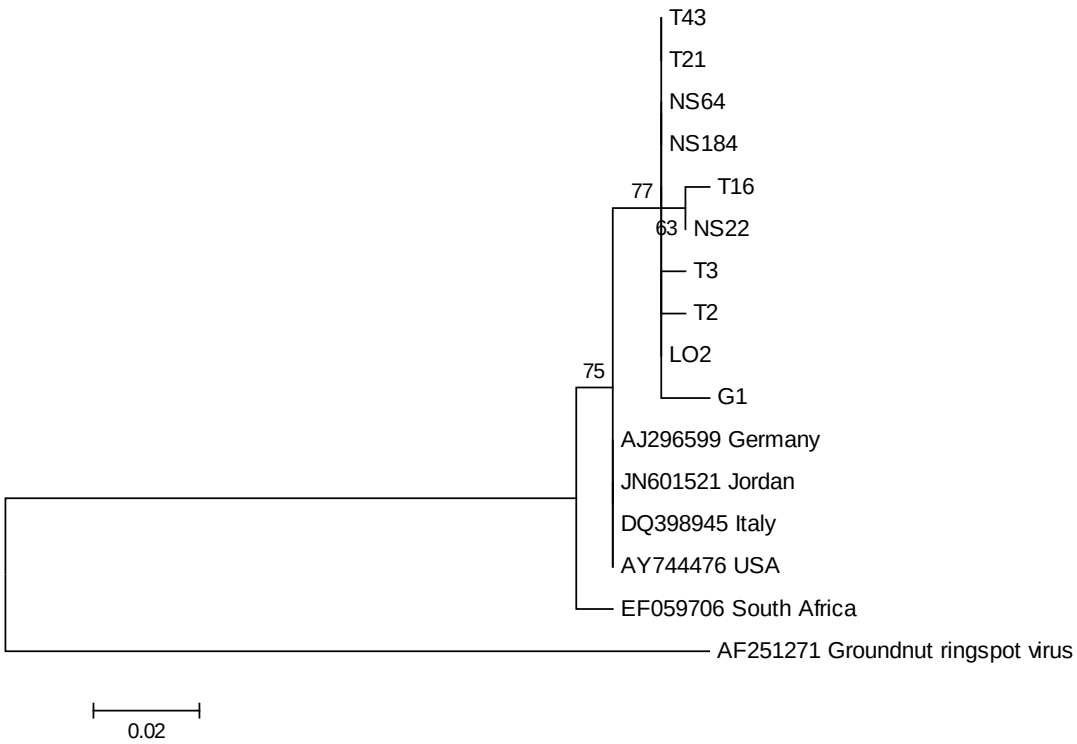

Fig. A2. Phylogenetic tree for partial N protein sequences from *Tomato spotted wilt virus* using the Maximum Likelihood method with 1000 bootstrap replicates based on the Kimura 2-Parameter model in MEGA 5. Isolates T2, T3, T16, T21 and T43 were extracted from thrips species while those labelled NS (Nakuru), LO (Loitokitok) and G (greenhouse) were extracted from tomatoes in Kenya. Representative databased sequences have been included.

Appendix 3. Supplementary data for Chapter 5

Table A3. Density of weed species per square metre within farms in four tomato production areas in Kenya

Botanical name	Nakuru	Kirinyaga	Loitokitok	Bungoma
Acanthospermum hispidum	-	7.1	-	-
Achyranthes aspera	-	-	-	3.0
Amaranthus hybridus	10.5	4.1	5.4	3.9
Amaranthus retroflexus	-	1.6	-	-
Amaranthus spinosus	-	9.6	-	-
Bidens pilosa	11.5	3.6	9.3	6.9
Brassica napus	2.4	1.1	0.9	1.3
Capsella bursa-pastoris	-	6.2	-	-
Chenopodium album	0.6	0.5	3.2	0.8
Cleome gynandra	0.6	1.3	0.8	1.3
Commelina benghalensis	1.9	1.8	2.0	3.2
Crotalaria polysperma	0.7	0.4	2.6	0.6
Cynodon dactylon	-	3.1	-	-
Cynoglossum coeruleum	-	-	-	-
Datura ferox	-	1.2	-	-
Datura stramonium	2.0	0.5	1.4	0.9
Digitaria scalarum	1.6	1.4	1.8	4.4
Eleusine indica	0.6	1.3	-	-
Fallopia convolvulus	1.4	-	6.4	-
Galinsoga parviflora	9.5	6.4	14.3	23.2
Ipomoea purpurea	0.4	1.6		0.2
Lactuca serriola	2.4	0.5	-	0.6
Lantana camara	-	1.2	-	3.6
Leonotis nepetifolia	0.6	-	3.6	-
Malva parviflora	0.8	0.5	1.0	2.5
Medicago sativa	-	4.6	-	-
Nicandra physalodes	1.0	1.0	1.1	0.9
Oxalis corniculata	0.8	-	-	1.2
Oxalis latifolia	14.0	6.9	-	11.1
Oxygonum sinuatum	0.8	1.2	1.2	2.4
Portulaca oleracea	4.8	2.9	-	-
Richardia brasiliensis	-	6.8	-	-
Senecio vulgaris	-	1.0	0.6	0.3
Setaria verticillata	-	0.8	-	-
Solanum incanum	0.2	0.6	0.4	0.8
Solanum nigrum	1.3	0.7	1.7	1.4
Sonchus oleraceus	1.0	0.4	1.6	0.8
Tagetes minuta	2.9	2.9	4.1	4.4
Tithonia diversifolia	-	0.4	-	-
Trifolium repens	-	-	-	0.8

Appendix 4. Supplementary data for Chapter 6

Table A4. Summary of tomato cultivars, their source, disease resistance and evaluation for Sw-5 gene in tomato cultivars screened for TSWV resistance

Variety	Plant Source[1]	Disease resistance[2]	ELISA	Daniese et at 2010	Shi et al 2011	
				Polymorphic primer*	Primer	SNPs
Claudia	Amiran	+bfg	Positive	Negative	-	
Sandokan	Royal	+afglm	Positive	Positive	+	
Gold Nugget	Starke Ayres	+defgm	Positive	Negative	-	
Cal J	East African	-	Positive	Negative	-	
Rambo	Royal	+fgkm	Positive	Negative	-	
Oxly premium	Royal	+fgj	Positive	Negative	-	
Mavuno	Royal	+d,f,k,m	Positive	Negative	-	
Money maker	Simlaw	-	Positive	Negative	-	
Nouvelle	Simlaw	-	Positive	Negative	-	
Heinz	Simlaw	-	Negative	Negative	-	
Marglobe	Simlaw	-	Positive	Negative	-	
Chonto	Royal	+b,c,d,f,g,h,m	Negative	Negative	-	
Fortune Maker	Simlaw	-	Positive	Negative	-	
Nuru	East African	-	Positive	Negative	-	
Little wonder	Starke Ayres	-	Negative	Negative	-	
Xewel	Savannah	+bef	Positive	Negative	-	
Heatmaster	Monsanto	+dfghjm	Negative	Negative	-	
Oxly	Royal	+d	Positive	Negative	-	
Roma	Starke Ayres	+fg	Positive	Negative	-	
M82	Simlaw	+fg	Positive	Negative	-	
VeloZ	Monsanto	+afgjm	Negative	Positive	+	
Dominator	Monsanto	+b	Positive	Negative	-	
Loreto	Monsanto	+fgjm	Negative	Negative	-	
Prostar	Simlaw	+bk	Positive	Negative	-	
Eden	Monsanto	-	Positive	Negative	-	
AB2	Monsanto	-	Positive	Positive	+	
Shanty	Amiran	+adfgm	Negative	Positive	+	
Tylka F1	Sygenta	+bdfgim	Positive	Negative	-	
Nema 1400	Monsanto	+fgm	Positive	Negative	-	
Kilele F1	Sygenta	+bdfgm	Positive	Negative	-	
Picus	Monsanto	+afghj	Negative	Positive	+	
Alex	Monsanto	+	Positive	Negative	-	
Jaguar	Savannah	+bf	Positive	Negative	-	
Ganila	Savannah	+fe	Negative	Negative	-	
Riograde	Simlaw	+fg	Positive	Negative	-	
Lucy F1	Savannah	+f	Negative	Negative	-	
Kiara F1	Savannah	+bf	Positive	Negative	-	
Tima	Savannah	+g	Negative	Negative	-	
Nemonetta	Amiran	+fgem	Negative	Negative	-	
DRG	Monsanto	+a	Negative	Positive	+	
Lindo	Savannah	+efk	Positive	Negative	-	
Bravo	Royal	+b,f,g,h,m	Negative	Negative	-	