Offered Review

AN AFRICAN PERSPECTIVE ON TOSPOVIRUSES

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SUMMARY

Tospoviruses, an emerging group of plant viruses, infect food crops and ornamental plants causing billions of dollars in losses worldwide. There are at least 28 *Tospovirus* species worldwide, most of which are found in tropical and subtropical environments. Despite having a largely tropical climate, Africa has only five reported species namely: *Tomato spotted wilt virus* (TSWV), *Groundnut ringspot virus* (GRSV), *Impatiens necrotic spot virus* (INSV), Tomato yellow ring virus (TYRV) and *Iris yellow spot virus* (IYSV). The low tospovirus diversity on the continent is mainly due to lack of surveys. Most *Tospovirus* species have only been reported in the last twenty-five years, yet no surveys have been done in most African countries. Most African countries lack institutional and infrastructural capacities to carry out virology research. This article reviews the characteristics and control of tospoviruses, with a bias towards those found in Africa.

Keywords: *Bunyaviridae*, diagnosis, host range, integrated disease management, nucleocapsid protein, thrips.

INTRODUCTION

Tospoviruses are amongst the most damaging and widespread emerging plant viruses worldwide, causing over US$ 1 billion losses annually in food and ornamental crops grown in greenhouses and open fields (Parrella *et al*., 2003). They belong to the genus *Tospovirus* within the family *Bunyaviridae*. They are the only plant-infecting viruses in this family as the other genera (*Hantavirus*, *Phlebovirus*, *Orthobunyavirus* and *Nairovirus*) contain animal-infecting viruses (Tsompana *et al*., 2005). They occur on all continents except Antarctica. At least one *Tospovirus* species is recorded in more than 60 different countries worldwide.

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The *Tospovirus* genus derives its name from its first and most studied member, *Tomato spotted wilt virus* (TSWV). TSWV was first reported in Victoria, Australia in 1915 (Brittlebank, 1919). Until the early 1990s, TSWV was the only species in the genus. However, there has been a proliferation of new species worldwide attributed to improved diagnostics, increased global trade in plants and plant products, climate change, rapid mutations and the occurrence of resistance-breaking strains (Hoffmann *et al*., 2001; Elliot, 2009). To date, there are 11 ICTV-recognized and at least 17 tentative species worldwide (Torres *et al*., 2012; Shimomoto *et al*., 2014; Rotenberg *et al*., 2015; Zheng *et al*., 2016). More new species continue to be reported worldwide, especially in Asia and South America.

This article provides an update on the status of tospoviruses in Africa, broadly outlining their classification, biological and physico-chemical properties, detection and diagnosis, vectors and control. It also attempts to explain the low tospovirus diversity on the continent when compared to other continents with similar climatic and agricultural patterns. Most of the tospoviral biological and physico-chemical properties described in this article are based on studies done on TSWV, the type species of the *Tospovirus* genus.

PROPERTIES AND CLASSIFICATION OF TOSPOVIRUSES

Tospoviruses have unique quasi-spherical particle morphology of 80-120 nm diameter. The *Tospovirus* virion has 5% nucleic acid, 5% carbohydrates, 20% lipids and 70% proteins (Adkins, 2000; Mukhopadhyay, 2011). It displays surface glycoprotein projections of 5-10 nm which are embedded in a lipid bilayer envelope 5 nm thick. It also has nucleocapsids that are 2-2.5 nm in diameter, and 200-300 nm long (Whitfield *et al*., 2005; Dong *et al*., 2008).

The *Tospovirus* genome consists of three single-stranded RNAs: one negative-sense large (L) RNA and two ambisense medium (M) and small (S) RNAs (King *et al*., 2012). The L RNA (*ca*. 8.9 kb) encodes for the RNA-dependent RNA polymerase (RdRp) in the viral complementary strand. The M and S segments each encode two proteins. The M RNA (*ca*. 4.8 kb) encodes the precursor to the envelope membrane glycoprotein in the viral complementary

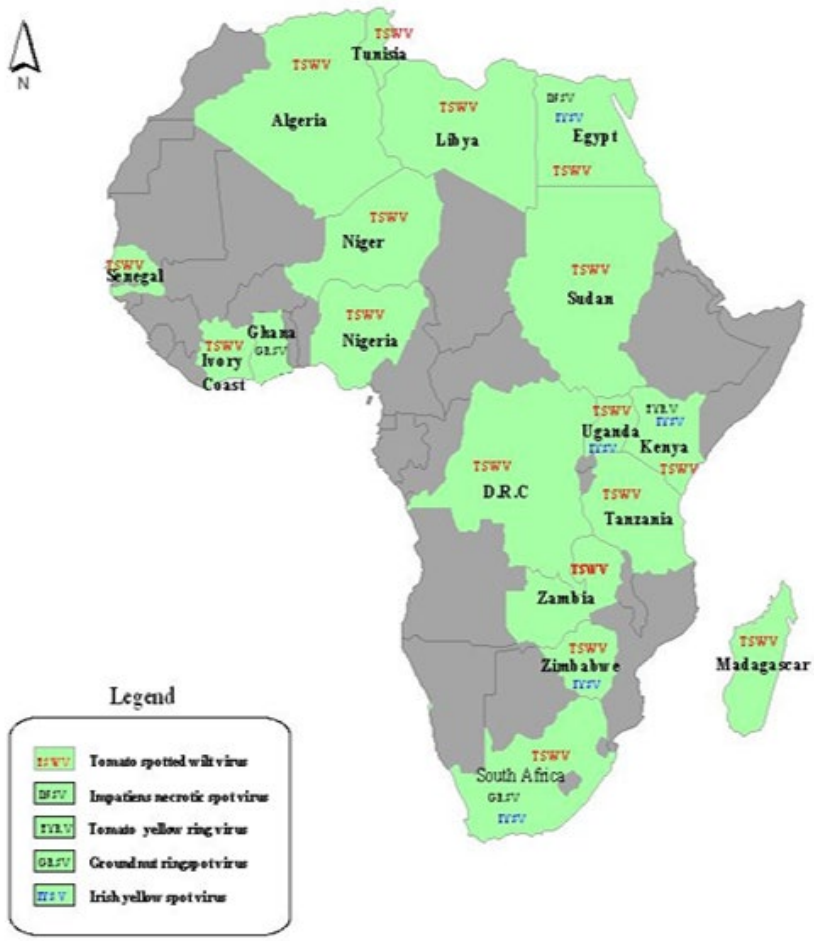

Fig. 1. Geographical distribution of tospoviruses in Africa.

(vc) sense, and the viral movement protein (NSm) in the viral (v) sense. The NSm is essential for systemic infection of plants by tospoviruses. The S RNA (*ca.* 2.9 kb) codes for the nucleoprotein (N) in the vc sense and the nonstructural protein (NSs) in the v sense (De Haan *et al*., 1990). The NSs behaves as a gene-silencing suppressor to counteract plant innate defense (Margaria *et al*., 2007). It also has a bi-function as ATPase and phosphatase, as shown from *Groundnut bud necrosis virus* (Lokesh *et al*., 2010). In *Frankliniella occidentalis*, Margaria *et al.* (2014) noted that the NSs protein is necessary for TSWV transmission and persistent infection. The N protein tightly associates with genomic RNA and, together with small amounts of viral RNA-dependent RNA polymerase (RdRp), form transcriptionally active ribonucleoproteins, the templates for RNA synthesis by RdRp (Geerts-Dimitriadou *et al*., 2012).

Tospovirus species are demarcated by host range, serology, vector specificity, genome structure and organization, and molecular relationships of N genes (Dong *et al*., 2008; Pappu *et al*., 2009; King *et al*., 2012). Serologically, tospoviruses were formerly subdivided into serogroups I through V, based on how species reacted to N protein antisera (McMichael *et al*., 2002). The N protein sequence is now the primary basis for species demarcation, with N protein identity of at least 90% denoting members of the same species, and N protein identity of less than 80% denoting distinct species (Fauquet *et al*., 2005). Viruses with 80-89% N protein identity may be considered as different strains or different species depending on their biological properties that include host range and vector thrips (Chiemsombat *et al*., 2008; Mahy and van Regenmortel, 2008).

TOSPOVIRUSES IN AFRICA

The first tospovirus-like disease from Africa was described in South Africa in 1905. This tobacco disease, referred to as "Kromnek" and later identified as tomato spotted wilt disease, was shown to be transmitted by *Thrips tabaci* and *Frankiliniella schultzei* (Thompson and van Zijl, 1996). To date, there are five reported tospoviruses namely: TSWV, *Impatiens necrotic spot virus* (INSV), *Groundnut ringspot virus* (GRSV), *Iris yellow spot virus* (IYSV) and Tomato yellow ring virus (TYRV). The geographical distribution of African tospoviruses is shown in Fig. 1.

Tomato spotted wilt virus. TSWV remains the most widespread of the reported *Tospovirus* species on the

continent. Besides South Africa, TSWV has been reported in Algeria, Cote d'Ivoire, the Democratic Republic of Congo, Egypt, Kenya, Libya, Madagascar, Mauritius, Niger, Nigeria, Reunion, Uganda, Senegal, Sudan, Tanzania, Tunisia, Uganda, Zambia and Zimbabwe (Ben Moussa *et al*., 2000, 2005; EPPO, 2004) (Fig. 1). There are unconfirmed reports of its presence in Morocco (EPPO, 2004). In South Africa, the virus occurs in seven out of nine provinces and is prevalent in the Eastern and Western Cape provinces where it seriously limits tomato and pepper production (Thompson and van Zijl, 1996; Sivparsad and Gubba, 2008). The earliest record of spotted wilt in Zimbabwe was by Hopkins (1940) in the Salisbury (now Harare) and Bulawayo districts. Kenya is the latest African country to report TSWV occurrence (Wangai *et al*., 2001), where it is currently confined to Nakuru Province (Macharia *et al*., 2015a).

The reported natural hosts of TSWV on the continent include flowers or ornamental plants, vegetables, field crops and weeds (Hean, 1938; Rothwell *et al*., 1982; Masuka *et al*., 1998; Dobson *et al*., 2002; Nyamupingidza and Machakaire, 2003; Macharia *et al*., 2016; Table 1). Most of these hosts belong to the families Asteraceae and Solanaceae. Recently, the virus was detected in butternut squash (*Cucurbita moschata* Duch.) in Zimbabwe (Karavina *et al*., 2016b), and this is the first record of TSWV infecting cucurbits on the continent. The pathogen has a narrower reported host range in Africa when compared to the over 1090 plant species reported worldwide (Parella *et al*., 2003). This can be attributed in part to the lack of comprehensive and recent host range studies in most African countries.

TSWV symptoms vary depending on host/plant species, cultivar, plant age, environmental conditions and host nutritional status, and virus isolate (Sether and DeAngelis, 1992). Common symptoms include ringspots, line patterns, mottling and chlorotic blotches on leaves. In tomato (*Solanum lycopersicum* L.), early plant infection causes severe stunting and even death. When young plants are infected, there is inward cupping of leaves. The leaves then develop a bronze cast followed by dark spots. As infection progresses, additional symptoms develop which include dark streaks on the main stem and wilting of the top portion. Fruits become deformed, show uneven ripening and raised bumps on the surface (Sether and DeAngelis, 1992). In infected pepper (*Capsicum annuum* L.) plants, severe stunting of young plants, and chlorotic or mosaic yellow flecking of leaves are observed. Necrotic spots are also present on pepper fruits, which often display ring patterns (Turina *et al*., 2012).

There are no records of the incidence, severity and economic impact of TSWV in Africa. Even though the virus is reported in 19 countries, there are no detailed records of how diagnoses were done except in South Africa and Kenya (Sivparsad and Gubba, 2008; Macharia *et al*., 2015a). To date, several African TSWV isolates have been partially characterized and the sequences deposited into GenBank. A phylogenetic analysis of these partial N gene sequences is shown in Fig. 2. Isolates from Algeria, Egypt, Kenya and Zimbabwe clustered in the same group (A), while those from South Africa were in a distinct group from the other African isolates. This may indicate different evolutionary pathways between the South African (in Group A) and the rest of African TSWV isolates (in Group B).

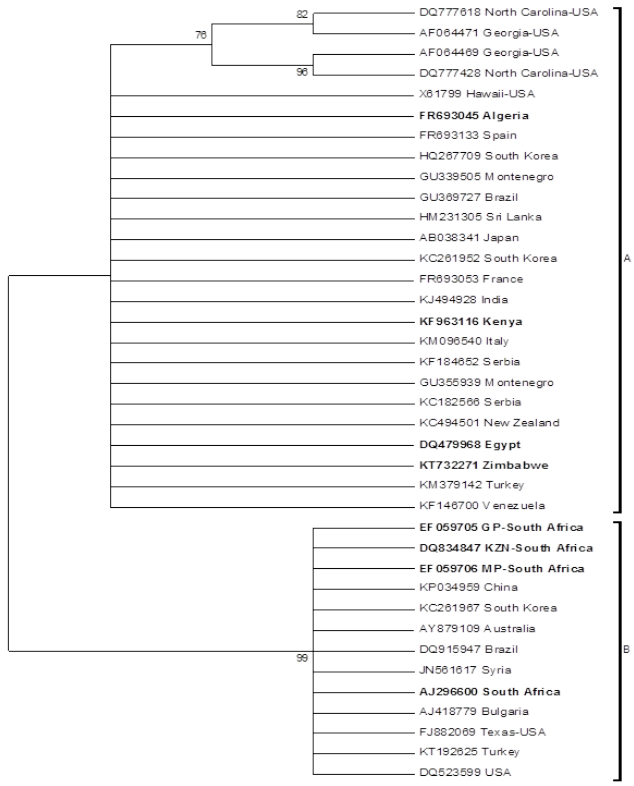

Fig. 2. Minimum-Evolution phylogenetic tree based on nucleotide sequences of the partial N gene of 38 TSWV isolates from different regions of the world. African TSWV isolates are in bold font. The tree was constructed with MEGA6 using the Close-Neighbor-Interchange algorithm. Bootstrap values greater than 75% are shown at the nodes based on 2000 replications.

Iris yellow spot virus. IYSV-like symptoms were first observed in onion (*Allium cepa* L.) plants in the Reunion Islands in 2003. The following year, IYSV was detected firstly in onion, and later in leek (*A. porrum*), garlic (*A. sativum*) and shallot (*A. cepa* var. *ascalonicum*) (Robene-Soustrade *et al*., 2005). Since then, the virus has been reported in *Allium sp.* in South Africa (du Toit *et al*., 2007), Mauritius (Lobin *et al*., 2012), Kenya and Uganda (Birithia *et al*., 2011), Egypt (Hafez *et al*., 2012) and Zimbabwe (Karavina *et al*., 2016a). Besides *Allium sp*., IYSV has been reported to naturally infect cabbage (*Brassica oleracea* var. *capitata*), snap pea (*Pisum sativum*), fat-hen *(Chenopodium album*), thorn-apple *(Datura stramonium*) and redroot pigweed *(Amaranthus retroflexus*) in Kenya (Birithia, 2013).

Table 1. *Tomato spotted wilt virus* host range in Africa.

Family	Species	Source
Amaranthaceae	*Ammaranthus hybridus*	Macharia *et al.* (2016)
	A. spinosus	Macharia *et al.* (2016)
	A. retroflexus	Macharia *et al.* (2016)
	Chenopodium album	Macharia *et al.* (2016)
Amaryllidaceae	*Agapanthus umbellatus*	Rothwell (1982)
Apiaceae	*Ammi majus*	Rothwell (1982)
Araceae	*Colocasia esculenta*	Rothwell (1982); Masuka *et al.* (1998)
	Dieffenbachia sp.	Masuka *et al.* (1998)
Asteraceae	*Bidens pilosa*	Rothwell (1982); Nyamupingidza and Machakaire (2003)
	B. subulternans	Macharia *et al.* (2016)
	Acanthospermum hispidum	Macharia *et al.* (2016)
	Callistephus chinensis	Rothwell (1982)
	Chrysanthemum morifolium	Hean (1938); Masuka *et al.* (1998)
	Coleopsis lanceolata	Rothwell (1982); Masuka *et al.* (1998)
	Dahlia pinnata	Hean (1938); Rothwell (1982)
	Dimorphotheca sp.	Rothwell (1982)
	Garlinsoga parviflora	Macharia *et al.* (2016)
	Lactuca serriola	Macharia *et al.* (2016)
	Leucanthemum maximum	Rothwell (1982)
	Senecio vulgaris	Macharia *et al.* (2016)
	Sonchus oleraceus	Macharia *et al.* (2016)
	Tagetes minuta	Macharia *et al.* (2016)
	Tithonia diversifolia	Macharia *et al.* (2016)
	Zinnia elegans	Hean (1938); Rothwell (1982)
Balsaminaceae	*Impatiens balsamina*	Rothwell (1982); Masuka *et al.* (1998)
Boraginaceae	*Cynoglossum coeruleum*	Macharia *et al.* (2016)
Brassicaceae	*Brasicca napus*	Macharia *et al.* (2016)
	Capsella bursa-p astoris	Macharia *et al.* (2016)
Campanulaceae	*Campanula persicifolia*	Hean (1938); Rothwell (1982)
Convolvulaceae	*Ipomea purpurea*	Macharia *et al.* (2016)
Cucurbitaceae	*Cucurbita moschata*	Karavina *et al.* (2016b)
Euphorbiaceae	*Euphorbia heterophylla*	Macharia *et al.* (2016)
Fabaceae	*Trifolium repens*	Macharia *et al.* (2016)
Geraniaceae	*Pelagonium peltatum*	Rothwell (1982)
Lamiaceae	*Physostegia virginiana*	Hean (1938); Rothwell (1982)
Malvaceae	*Malva parviflora*	Macharia *et al.* (2016)
Pandanaceae	*Pandanus utilis*	Masuka *et al.* (1998)
Papaveraceae	*Papaver nudicaule*	Hean (1933)
Plantaginaceae	*Pentstemon sp.*	Hean (1933); Rothwell (1982)
Poaceae	*Cynodon dactylon*	Macharia *et al.* (2016)
Polemoniaceae	*Phlox drummondii*	Hean (1938)
Polygonaceae	*Fallopia convolvulus*	Macharia *et al.* (2016)
Primulaceae	*Primula obconica*	Rothwell (1982)
Portulacaceae	*Portulaca oleracea*	Macharia *et al.* (2016)
Rubiaceae	*Richardia brasiliensis*	Macharia *et al.* (2016)
Solanaceae	*Browallia speciosa*	Rothwell (1982)
	Brunfelsia pauciflora	Rothwell (1982)
	Capsicum annuum	Masuka *et al.* (1998)
	Capsicum frutescens	Rothwell (1982)
	Cestrum aurantiacum	Rothwell (1982)
	Datura ferox	Macharia *et al.* (2016)
	D. stramonium	Hean (1938); Macharia *et al.* (2016)
	Nicandra physalodes	Macharia *et al.* (2016)
	Nicotiana tabacum	Hean (1938); Masuka *et al.* (1998)
	Physalis peruviana	Hean (1938); Rothwell (1982)
	P. angulata	Macharia *et al.* (2016)
	Solanum chenopodiodes	Macharia *et al.* (2016)
	S. lycopersicum	Rothwell (1982); Masuka *et al.* (1998); Nyamupingidza and Machakaire (2003)
	S. nigrum	Macharia *et al.* (2016)
	S. tuberosum	Hean (1938); Masuka *et al.* (1998)
	Petunia violacea	Rothwell (1982); Masuka *et al.* (1998)
Tropaeolaceae	*Tropaeolum majus*	Hean (1938); Rothwell (1982)
Verbanaceae	*Lantana camara*	Macharia *et al.* (2016)
Violaceae	*Viola odorata*	Rothwell (1982)

Infected alliaceous plants display a wide range of symptoms. Early symptoms display yellow to straw-colored chlorotic or necrotic lesions, elongated, lenticular or spindle-shaped, from the middle to the lower portion of the infected plant. As the disease develops and plant grows, lesions elongate and coalesce, covering the leaves and scapes. Sometimes, green tissue islands appear in the centre of a diamond-shaped lesion (Bag *et al*., 2015).

High IYSV disease incidences of at least 60% and severities of 75% were reported in onions in Mauritius (Lobin *et al*., 2012), Kenya (Birithia *et al*., 2014) and Zimbabwe (Karavina *et al*., 2016a). In the other countries where IYSV is present, incidence and severity figures are not documented. In Kenya, Birithia *et al*. (2014) noted that onion varieties resistant to *Thrips tabaci* infestation had low IYSV incidence. Such varieties subsequently produced higher yields when compared to susceptible varieties. The economic impact of IYSV in most African countries is yet to be determined. However, the pathogen is capable of causing up to 100% yield loss in onion seed and bulb crops in other regions of the world (Pozzer *et al*., 1999).

To date, there are no complete genome sequences of African IYSV isolates. However, based on partial N gene sequences, all African IYSV isolates are closely related to each other and clustered in the same group (Fig. 3). Isolates from Iran, Serbia, The Netherlands and UK were in the other group.

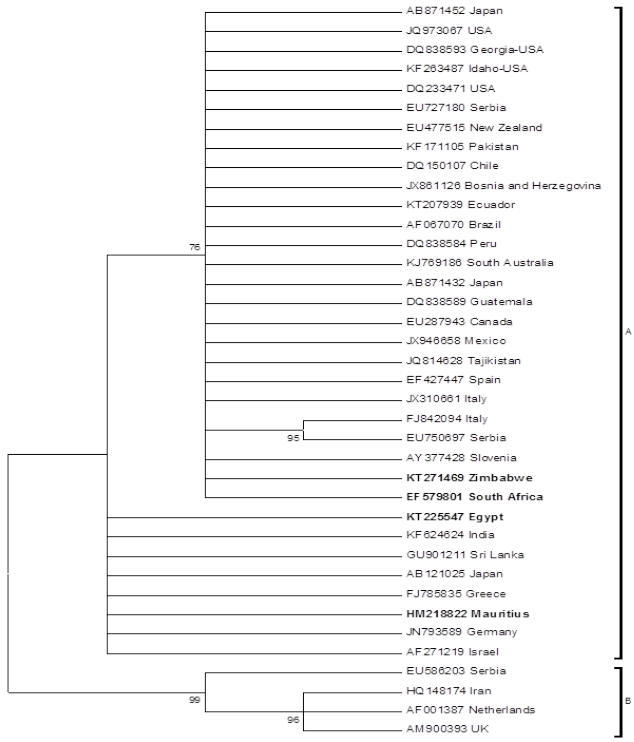

Fig. 3. Minimum Evolution tree of the partial N gene region of African IYSV isolates (in bold font) in relation to those found elsewhere in the world. The bootstrap consensus is based on 2000 replications. The evolutionary distances were computed using the Tamura 3-parameter method. Evolutionary analyses were conducted in MEGA6.

Groundnut ringspot virus. GRSV has been reported in South Africa and Ghana. In South Africa, GRSV was first reported in groundnuts (*Arachis hypogeae* L.) (reviewed in Pappu *et al*., 2009), and then in soybean (*Glycine max* L.) (Pietersen and Morris, 2002), while in Ghana, it has recently been reported co-infecting groundnuts with the groundnut rosette disease (Appiah *et al*., 2016). There are no records of GRSV distribution, incidence and severity in South Africa, but in Ghana, viral infection rates of up to 69.5% were reported (Appiah *et al*., 2016).

While the virus has two reported hosts in Africa, it also infects cucumber (*Cucumis sativus* L.) in Brazil (Spadotti *et al*., 2014), coriander (*Coriandrum sativum* L.), eggplant (*Solanum melongena* L.), pepper, tomato and tomatillo (*Physalis ixocarpa*) in Florida, USA (Webster *et al*., 2011). Chlorotic ringspots and line-patterns are the common symptoms in groundnuts (Appiah *et al*., 2016). In pepper and tomato, chlorotic and necrotic spots on leaves, deformed leaves and fruits, necrosis of stems and terminal growing points are observed. Early infected tomato plant leaves roll inwards and develop a bronze cast followed by dark brown flecks. Fruits show uneven ripening, and often have raised bumps and/or ring patterns on the surface (Webster *et al*., 2011). GRSV symptoms on soyabean are not described.

Impatiens necrotic spot virus. On the African continent, INSV has only been reported in Egypt in 16 plant species that include ornamentals, field crops and weeds (El-Wahab *et al*., 2011). However, INSV has the second widest host range amongst the tospoviruses worldwide (Jones, 2005), with 648 species from 50 families (Wang and Guo, 2004). It is a serious pathogen of greenhouse ornamentals where up to 100% yield losses have been reported (Daughtrey *et al*., 1997). As with other tospoviruses worldwide, INSV host range is expanding (Perry *et al*., 2005).

Common INSV-induced symptoms include downward curling of leaves, leaf tip dieback, necrosis of growing tips, sunken chicken pox-like spots on leaves, leaf yellowing and ringspots, stunting and stem death (McMillan and Graves, 1999; Ding *et al*., 2011). There are no records on the severity and economic impact of the virus in Egypt.

Tomato yellow ring virus. The most recently reported tospoviral species on the continent is TYRV. This virus, reported from Kenya, was identified in tomatoes during TSWV surveys between March 2010 and January 2012 (Birithia *et al*., 2012). Infected tomato fruits develop either chlorotic ring spots or a bright yellow ring pattern, while leaves and stems become necrotic. Plants generally show stunted growth (Winter *et al*., 2005; Beikzadeh *et al*., 2012). No information on TYRV disease incidence, severity and impact on yield is available.

Table 2. Thrips vectors of *Tospovirus* species occurring in Africa.

Tospovirus spp.	Thrips vector	Natural hosts	References
GRSV	Unknown	Groundnut, soybean	Pietersen and Morris (2002).
INSV	*F. occidentalis*	Ornamental plants	Ben Moussa *et al.* (2005).
IYSV	*T. tabaci*	*Allium spp*	Hafez *et al.* (2012); Lobin *et al.* (2012).
TSWV	*F. occidentalis*, *F. schultzei*, *T. Tabaci*	Many plants	Ben Moussa *et al.* (2000).
TYRV	Unknown	Tomato	Birithia *et al.* (2012).

Worldwide, TYRV has a narrow host range. Besides tomato, it also infects soybean (*Glycine max* L.), potato and ornamentals (Hassani-Mehraban *et al.*, 2005; Beikzadeh *et al.*, 2012). The geographical range of TYRV is expanding, with the virus also present in Iran (Hassani-Mehraban *et al.*, 2005) and Poland (Zarzynska-Nowak *et al.*, 2016).

Taxonomically, TYRV clusters in a clade with IYSV and Polygonum ringspot virus (Hassani-Mehraban *et al.*, 2005). The Kenyan TYRV isolate (GenBank Accession No. JQ955615) revealed at least 98% N sequence identity with TYRV isolates infecting *Cineraria* (DQ788694), *Anemone* (DQ788693) and *Alstroemeria* (HQ154130), and tomato-infecting Tomato yellow fruit ring virus (TYFRV) isolates (AJ493270.1, AY686718.1 and HQ154131.1) from Iran (Birithia *et al.*, 2012).

DIVERSITY AND DISTRIBUTION OF *TOSPOVIRUS* VECTORS IN AFRICA

Tospoviruses are transmitted exclusively by thrips (Thysanoptera: Thripidae) in a circulative-propagative mode (Moritz *et al.*, 2004). There are over 7 000 thrips species worldwide (Rebijith *et al.*, 2014), of which 1% are plant pests. Only 15 thrips species are known to naturally transmit tospoviruses. These belong to the genera *Frankliniella* (8), *Thrips* (3), *Ceratothripoides* (1), *Dictyothrips* (1), *Neohydatothrips* (1) and *Scirtothrips* (1) (Rotenberg *et al.*, 2015). Of these, *Frankliniella occidentalis* (Pergande), *Frankliniella schultzei* Trybom, *Thrips tabaci* Lindeman, *Thrips palmi* Karny and *Scirtothrips dorsalis* Hood are present in Africa (Timm *et al.*, 2008; Macharia *et al.*, 2015b; CABI, 2016a, 2016b, 2016c; Table 2).

Frankliniella occidentalis, also known as the western flower thrips (WFT), originated in the western USA, but has now spread throughout the world through international shipments of ornamental plants (Kirk and Terry, 2003). In Africa, the WFT was first reported in South Africa in 1987 (Giliomee, 1989), and is now present in Algeria, Egypt, Kenya, Morocco, Reunion, Swaziland, Tunisia, Uganda and Zimbabwe (CABI, 2016a). It has a worldwide host range of over 200 plant species from more than 60 families including ornamentals, vegetables and weeds (Jones, 2005). The warm climate on the African continent is ideal for WFT survival. This species transmits up to seven tospoviruses worldwide (Rotenberg *et al.*, 2015), and is a confirmed vector of TSWV and INSV in Africa (Abdelkader *et al.*, 2005; El-Wahab *et al.*, 2011).

Thrips tabaci Lindeman, another cosmopolitan species, is abundant in Africa because of the warm climatic conditions that prevail on the continent. It transmits IYSV, TSWV and TYFRV worldwide, but in Africa, it has been reported to transmit IYSV and TSWV (Abdelkader *et al.*, 2005; Birithia *et al.*, 2014). Both arrhenotokous and thelytokous populations of *T. tabaci* exist on the continent (Macharia *et al.*, 2015b).

Frankliniella schultzei (Trybom), commonly called the common blossom thrips, is also widespread throughout Africa. It is another polyphagous pest which transmits six tospoviruses worldwide (Rotenberg *et al.*, 2015). It is reported as a TSWV vector in South Africa (Thompson and van Zijl, 1996).

Scirtothrips dorsalis, a pest of South-East Asian origin, is another cosmopolitan pest, with a host range of 150 plant species and 40 families (Jones, 2005; Riley *et al.*, 2011). Worldwide, it transmits *Groundnut bud necrosis virus* (GBNV), Groundnut chlorotic fanspot virus (GCFSV) and *Groundnut yellow spot virus* (GYSV). All three *Tospovirus* species are prevalent in Asia, while GCFSV also occurs in South America (Pappu *et al.*, 2009). None of these tospoviruses have been reported in Africa to date. *Scirtothrips dorsalis* has so far been confirmed to be present in Cote d'Ivoire, Kenya and Uganda (Macharia *et al.*, 2015b; CABI, 2016b).

Thrips palmi is present in Cote d'Ivoire, Mauritius, Nigeria, Reunion and Sudan (CABI, 2016c). It transmits Calla lily chlorotic spot virus (Chen *et al.*, 2005), GBNV (Meena *et al.*, 2005), Melon yellow spot virus (MYSV) (Kato *et al.*, 2000) and *Watermelon silver mottle virus* (WSMoV) (Iwaki *et al.*, 1984) in Asia. None of these tospoviruses have been reported in Africa to date.

RAPID *TOSPOVIRUS* DETECTION TECHNIQUES AND NUCLEIC ACID STORAGE

Successful *Tospovirus* diagnosis is dependent upon the sampling technique employed. As with all RNA viruses, collected plant samples must be processed immediately to prevent deterioration. This is often difficult in most African countries due to the absence of well-equipped plant virology laboratories. So, techniques for quick virus detection and/or storage for later processing have to be employed. In several African countries, quick field detection of tospoviruses has been attained by using immunostrips, while long-term sample storage has been achieved by using

FTA® (Whatman) cards and RNA stabilization solutions or buffers.

Tospovirus immunostrips are lateral flow devices that have tospoviral antibodies impregnated within an absorbent pad. They can either detect specific or several tospoviruses. For example, the general immunostrips produced by Loewe® Biochemica Industries (Germany) can detect TSWV, Tomato chlorotic spot virus (TCSV) and GRSV, while ImmunoComb® Kit by Agdia Inc., (Elkhart, USA) can detect TSWV, INSV, *Cucumber mosaic virus* (CMV) and *Tobacco mosaic virus* (TMV). Immunostrips have been used in Kenya and Uganda for detecting IYSV (Birithia *et al*., 2011), and Zimbabwe for detecting TSWV (Karavina *et al*., 2016b).

Once tospoviruses have been detected by immunostrips, infected plant material can then be collected and stored in RNA stabilization buffers for later processing. The buffers are approved to preserve and protect viral nucleic acid in the plant samples for up to one day at 37°C, one week at room temperature, up to one month at 2-8°C, or indefinitely at or below –20°C. Examples of commercially available stabilization solutions are RNA*later*® (Qiagen) and RNA Shield™ (Zymo Research). *RNAlater*® preserves the infectivity of RNA viruses in samples stored at room temperature for up to 72 hours (Uhlenhaut and Kracht, 2005; Steward and Culley, 2010). It has been used extensively in viral surveys on the continent. RNA Shield™, on the other hand, has been used as an RNA stabilizer during transportation to analytical facilities which may be several hours or days away from extraction laboratories.

The FTA® cards are a paper-based technology designed for the collection and archiving of nucleic acids, either in their purified form or within pressed samples of fresh tissue. They have been used in Africa for both DNA and RNA plant viruses (Ndunguru *et al*., 2005). In Zimbabwe, Karavina *et al*. (2016b) used FTA cards to archive tospoviral nucleic acid. The card contains chemicals that lyse cells, denature proteins and protect nucleic acids from nucleases, oxidative and UV damage. Pressed samples can be stored at room temperature for up to month for RNA viruses and processed when required (Predajna *et al*., 2012). Viral RNA is eluted by a simple protocol and used as template for amplification by reverse transcription-polymerase chain reaction (RT-PCR), cloning and sequencing (Grund *et al*., 2010; Kouassi *et al*., 2010). Because RNA is highly unstable, nucleic acid elution must be done within four weeks if tospoviruses are to be recovered from FTA cards.

TOSPOVIRUS DIAGNOSIS

Several techniques, including host indexing, electron microscopy, serology and molecular assays, are used to characterize tospoviruses in Africa. Commonly used indicator hosts include *Nicotiana tabacum*, *N. benthamiana* and *Chenopodium quinoa*. Though it has high sensitivity, indexing may not identify the actual *Tospovirus* sp. and is time-consuming (Jan *et al*., 2012).

Electron microscopy is an expensive technique that requires highly technical and experienced staff for operation. Most African academic and research institutions do not have electron microscopes because they are expensive to purchase and maintain. However, electron microscopy has been used for TSWV diagnosis in South Africa (Sivparsad and Gubba, 2008).

A cheaper, easier and widely used diagnostic technique on the continent is serology. The most commonly used serological method is enzyme-linked immunosorbent assay (ELISA). Of the two ELISA variants commonly used in virology [double antibody sandwich- enzyme linked immunosorbent assay (DAS-ELISA) and triple antibody sandwich-ELISA], DAS-ELISA is widely used Africa. Commercial ELISA kits sourced from Europe and the USA have been used in the detection and diagnosis of IYSV and TSWV in Kenya, Mauritius, South Africa, Reunion and Zimbabwe.

Molecular assays are now widely employed in *Tospovirus* studies in Africa. Since tospoviruses are RNA viruses, reverse transcription polymerase chain reaction (RT-PCR) is employed for their diagnosis. In all cases, RT-PCR followed by partial N gene sequencing, have been used to confirm viral identities. In African countries without the infrastructure for molecular diagnosis, samples are sent to countries with the capacity to carry out the analyses (Robene-Soustrade *et al*., 2005; Karavina *et al*., 2016b). Though no full genome sequences of African *Tospovirus* isolates are available to date, facilities for Next Generation Sequencing (NGS) are available in South Africa. A major limitation with NGS is its high cost when compared to Sanger Sequencing.

MANAGEMENT OF TOSPOVIRAL DISEASES

It is very difficult to eradicate tospoviruses in regions where they have infected crops because of their wide host ranges and efficient transmission by polyphagous vectors (Riley *et al*., 2011). Control measures employed against tospoviruses must be based on thorough understanding of epidemiological principles. The measures should aim to control the vector, reduce carry-over of virus inoculum between crop cycles and reduce pathogen multiplication in a host (Jones, 2004). Information on specific *Tospovirus* control is generally lacking in most African countries. But, control methods from other regions with similar tospoviruses and climatic conditions can be adapted for the continent. These methods must fall within Integrated Disease Management (IDM) approaches that include biological, chemical, cultural, physical, host plant resistance and phytosanitary practices (Culbreath *et al*., 2003).

Biological control. Biocontrol relies on the use of thrips' natural enemies like the predatory mites

(*Amblyseius cucumeris*, *Neoseiulus cucumeris*, *Stratiolaelaps miles* and *Geolaelaps aculeifer*), the minute pirate bug (*Orius* sp.), parasitoids (*Ceranisus menes*), entomopathogenic nematode (*Steinerma feltiae*) and entomopathogenic fungi (*Beauveria bassiana* and *Metarhizium anisopliae*) to keep thrips populations down. A number of these biocontrol agents are present in Africa and are being employed in integrated pest management as part of sustainable farming practices. For example, in Kenya, *Ceranisus* and *Orius* sp. are used to control WFT in French beans (*Phaseolus vulgaris* L.), a high-value export crop (Nyasani *et al*., 2013, 2015). The International Centre for Insect Physiology and Ecology (ICIPE) has formulated biopesticides using *Metarhizium anisopliae* isolates. One such biopesticide is Campaign®, now registered in Ethiopia, Kenya, Ghana, South Africa and Tanzania for thrips control. However, these biocontrol agents and products are not being used to directly control thrips in *Tospovirus*-infected crops.

Biocontrol can be difficult to implement especially amongst smallholder farmers most of who tend to apply synthetic pesticides indiscriminately. It is perceived to be slow-acting and not ideal for short-season annual crops. The key to successful biocontrol is releasing the natural enemies early enough in the cropping cycle to allow them to build up their population to levels able to suppress thrips populations. If implemented as part of IDM, farmers should apply selective and non-persistent pesticides so as to preserve biocontrol agents.

Chemical control. Chemical control aims to reduce thrips vector population so as to reduce virus spread. Systemic insecticides are most ideal since thrips like to inhabit areas that contact insecticides cannot readily penetrate (Melzer *et al*., 2012). Both foliar and soil-applied insecticides have been used with some degree of success in managing thrips. Insecticides like imidacloprid, abamectin, carbamates, organophosphates and synthetic pyrethroids are widely used in agriculture on the continent (Dobson *et al*., 2002; Nyasani *et al*., 2015).

Thrips tend to quickly develop resistance to most insecticides used against them. Therefore, to achieve sustainable pest control, the application of newer insecticides and those insecticides with different chemistries and modes of action in an insecticide rotation system has to be done (Kay and Herron, 2010). Insecticide application should also be done judiciously so that there are no toxic residues in the environment and food produce meant for consumption (Pappu *et al*., 2009). Most of the newer insecticides are still patented and therefore more expensive to farmers than generic insecticides.

Physical and cultural control. The physical and cultural control tactics currently employed in tospoviral disease management include growing crops in protected environments, field hygiene, and adjusting crop planting dates. Screen meshes are placed on greenhouse air vents and sidewalls to reduce thrips entry into greenhouses. Farmers also hang yellow sticky traps above growing crops in greenhouses to trap thrips (Dobson *et al*., 2002). In the field, some farmers cover the ground with straw mulches thereby allowing the build-up of thrips predators.

Commonly practiced field hygienic measures include the destruction of old infected crops, volunteer plants, symptomatic and asymptomatic weeds at or around planting sites. Weeds in the Compositae and Solanaceae families and those with yellow flowers are very attractive to thrips (Blumthal *et al*., 2005) and so must be destroyed so that they do not act as "green bridges" in disease epidemiology.

Intercropping, manipulation of crop planting dates and plant densities, and crop rotation are also used extensively by farmers. In Kenya, Gachu *et al*. (2012) demonstrated that *T. tabaci* in bulb onions can be managed by intercropping with carrots (*Daucus carrota*) and spider plants (*Cleome gynandra*). Planting at high densities dilutes the overall numbers of infected plants and helps healthy plants to shade out neighboring infected plants (Brown *et al*., 1996). It also allows compensation for yield loss of diseased plants by adjacent healthy plants (Culbreath *et al*., 2003). Since disease build-up is greatly influenced by weather conditions, crops grown during the cool season suffer lesser damage than those grown in summer when both vector activity and virus multiplication are high. Farmers should adjust crop planting dates such that susceptible stages of crop development do not coincide with peak or increasing thrips populations.

Farmers should avoid overlapping sowing of susceptible crops and sequential plantings side by side. This tends to create a "green-bridge" as infected thrips vectors can migrate from the old crop to the new crop and spread the viruses. They should practice crop rotation and fallowing. Growing non-susceptible crops or establishing a three-week fallow period allows thrips to migrate out of an area earmarked for growing a susceptible crop (Cho *et al*., 1998). During the fallow period, greenhouses should be heated for 4-5 hours at 30°C daily (Cloyd, 2009) to speed up pupation of any overwintering pupae which then starve to death. It has been observed that sometimes farmers disregard all these disease management principles in pursuit of high profits offered by crops which may be highly susceptible to tospoviruses and their vectors.

Host plant resistance. The biological, chemical, cultural and physical methods employed in tospoviral disease management have only achieved limited success (Soler *et al*., 2003). Thus, the development of host plant resistance appears to be the most effective strategy of managing tospoviruses. In South Africa, breeding for TSWV resistance in tomatoes started in the late 1960s. This led to the release of the resistant "Stevens" cultivar in 1988 (Thompson and van Zijl, 1996). Resistance from this line is ensured by the *Sw-5b* gene and has been introgressed into most processing and fresh market tomato cultivars currently being

produced in South Africa and marketed in 55 countries worldwide. However, this resistance is not durable, and there have been TSWV resistance-breaking strains in South Africa (Lopez *et al*., 2011).

With IYSV being a threat to bulb onion production in Kenya, screening of commercial onion varieties was initiated (Birithia *et al*., 2014). Some varieties like "Texas Grano" supported low thrips populations, and have been recommended for production in regions with high IYSV disease pressure. In Ghana, the high GRSV incidence has necessitated the initiation of breeding programs for disease management.

Despite the limited utility of natural resistance, and the long duration required to breed resistant plants, there hasn't been any genetically-engineered *Tospovirus*-resistant crops grown in Africa. Major contributors to this are the socio-political debate surrounding the adoption of genetically-modified plants in most countries, and the costs associated with transgenic breeding.

WHY SO FEW REPORTED *TOSPOVIRUS* SPECIES IN AFRICA?

When compared to other tropical and subtropical regions of the world, Africa has a low *Tospovirus* diversity (Pappu *et al*., 2009). Africa has only five reported species, while Asia and South America have at least 21 and 11 species, respectively. The few reported species in Africa is partly due to lack of recent *Tospovirus* surveys in most countries. Only Egypt, Kenya, Mauritius, Uganda, Reunion Islands, South Africa, Tunisia and Zimbabwe have reported tospoviral studies in the last two decades.

Since the 1990s, there has been a proliferation of new *Tospovirus* spp. worldwide. This has been attributed to climate change, increased global trade in plants and plant products, improved virus diagnostics, rapid mutations and the occurrence of resistance-breaking strains. Some species previously classified as TSWV were reclassified as new species when molecular assays were employed in tospovirus diagnostics (Law and Moyer, 1990). There is need for up-to-date surveys to be carried out using modern diagnostic techniques so as to ascertain the current status of tospoviruses on the continent. The fact that recent surveys in Egypt, Ghana, Kenya and Zimbabwe have unearthed tospoviruses that were previously undocumented in these countries further supports the call for more surveys on the continent. There is a lot of uncontrolled trade in plant and plant products in Africa, and a *Tospovirus* like IYSV most likely has a wider geographical distribution than has been reported. For example, the IYSV detected in Zimbabwe's Manicaland Province (Karavina *et al*., 2016c), was at a farm close to the border with Mozambique. Yet, there are currently no IYSV reports in Mozambique, despite the high levels of uncontrolled agricultural trade between border communities living in both countries.

Another major contributor to the few *Tospovirus* studies is the lack of institutional and infrastructural capacities by the majority of research and training institutions in most African countries. The training of plant virologists is much more expensive than the training of bacteriologists, mycologists and nematologists. The equipment and consumables needed for plant virology studies are very expensive. Most research and training institutions in Africa lack even the basic facilities for plant virology research. Even the retention of trained plant virologists is difficult because of low remuneration.

The lack of information on *Tospovirus* disease incidence, severity and economic impact in most African countries is a cause for concern given the devastation these viruses cause worldwide. In Asia, GBNV causes US$89 million losses per year (Mandal *et al*., 2012). In Hawaii, farmers had to abandon tomato production due to serious TSWV infection (Cho *et al*., 1998). These regions have similar climatic and agricultural patterns to Africa, yet the economic impact of tospoviruses on the continent remains largely known.

Recently, there seems to be interest in studying tospoviruses on the continent. This has resulted in the identification and characterization of new *Tospovirus* sp., their hosts and vectors, and the development of sustainable farming practices adaptable to the continent (Macharia *et al*., 2015b; Nyasani *et al*., 2015). If these studies can be replicated throughout the continent, then the effects and distribution of tospoviruses will become clearer. As of now, the true story on the diversity, incidence, severity and economic impact of African tospoviruses remains largely unknown.

REFERENCES

Abdelkader H.S., Allam G.M., Moustafa T.A., EL-Hammady M.H., 2005. Characterization and molecular detection of *Tomato spotted wilt virus* (*Tospovirus*) infecting tomato in Egypt. *International Journal of Virology* **1**: 10-10.

Adkins S., 2000. *Tomato spotted wilt virus*- Positive steps towards negative success. *Molecular Plant Pathology* **1**: 151-157.

Appiah A.S., Offei S.K., Tegg R.S., Wilson C.R., 2016. Varietal response to Groundnut rosette disease and the first report of *Groundnut ringspot virus* in Ghana. *Plant Disease* **100**: 946-952.

Bag S., Schwartz H.F., Cramer C.S., Harvey M.J., Pappu H.R., 2015. *Iris yellow spot virus* (*Tospovirus: Bunyaviridae*): from obscurity to research priority. *Molecular Plant Pathology* **16**: 224-237.

Beikzadeh N., Bayat H., Jafarpour B., Rohani H., Peters D., Hassani-Mehraban A., 2012. Infection of *Alstraemeria* plants with Tomato yellow ring virus in Iran. *Journal of Phytopathology* **160**: 45-47.

Ben Moussa A., Makni M., Marrakchi M., 2000. Identification of the principle viruses infecting tomato crops in Tunisia. *OEPP/EPPO Bulletin* **30**: 293-296.

Ben Moussa A., Marrakchi M., Makni M., 2005. Characterization of *Tospovirus* in vegetable crops in Tunisia. *Infection, Genetics and Evolution* **5**: 299-322.

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

Birithia R., Subramanian S., Villinger J., Muthoni 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**: 1384.

Birithia R.K., 2013. Identification and distribution of thrips-vectored tospoviruses and management of *Iris yellow spot virus* in onion. PhD Thesis. University of Nairobi, Kenya.

Birithia R.K., Subramanian S., Muthomi J.W., Narla R.D., 2014. Resistance to *Iris yellow spot virus* and onion thrips among onion varieties grown in Kenya. *International Journal of Tropical Insect Science* **34**: 73-79.

Blumthal M.R., Cloyd R.A., Spomer L.A., Warnock D.F., 2005. Flower color preferences of western flower thrips. *HortTechnology* **15**: 846-855.

Brittlebank C.C., 1919. Tomato diseases. *Journal of Agriculture* **27**: 231-235.

Brown S.L., Todd J.W., Culbreath A.K., 1996. Effect of selected cultural practices on incidence of *Tomato spotted wilt virus* and populations of thrips vectors in peanuts. *Acta Horticulturae* **431**: 492-498.

CABI, 2016a. Invasive species compendium. *Frankliniella occidentalis* (western flower thrips). Centre for Agricultural Bioscience International. Available at: http:www.cabi.org/isc/datasheet/24426.

CABI, 2016b. Invasive species compendium. *Scirtothrips dorsalis* (chilli thrips). Centre for Agricultural Bioscience International. Available at: http:www.cabi.org/isc/datasheet/49065.

CABI, 2016c. Invasive species compendium. *Thrips palmi*. Centre for Agricultural Bioscience International. Available at: http:www.cabi.org/isc/datasheet/53745.

Chen C.C., Chen T.C., Lin Y.H., Yeh S.D., Hsu H.T., 2005. A chlorotic spot disease of calla lilies (*Zantedeschia* spp.) is caused by a *Tospovirus* serologically but distinctly related to *Watermelon silver mottle virus*. *Plant Disease* **89**: 440-445.

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**: 571-577.

Cho J., Mau R.F.L., Pang S-Z., Wang M., Gonsalves C., Watterson J., Custer D.M., Gonsalves D., 1998. Approaches for controlling *Tomato spotted wilt virus*. In: Hadidi A., Khetarpal R.K., Koganezawa H. (eds). Plant Virus Disease Control, pp. 547-564. APS Press, Minnesota, USA.

Cloyd R.A., 2009. Western flower thrips (*Frankliniella occidentalis*) management on ornamental crops grown in greenhouses: have we reached an impasse? *Pest Technology* **3**: 1-9.

Culbreath A.K., Todd J.W., Brown S.L., 2003. Epidemiology and management of Tomato spotted wilt in peanut. *Annual Review of Phytopathology* **41**: 53-75.

Daughtrey M., Jones R.K., Moyer J.W., Daub M.E., Baker J.E., 1997. Tospoviruses strike the greenhouse industry – INSV has become a major pathogen on flower crops. *Plant Disease* **81**: 1220-1230.

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**: 1000-1007.

Ding M., Yin Y., Fang Q., Li S., Zhang Z., 2011. First report of *Impatiens necrotic spot virus* in *Gentiana macrophylla* in China. *Plant Disease* **95**: 357.

Dobson H., Cooper J., Manyangarirwa W., Karuma J., Chiimba W., 2002. Integrated Vegetable Pest Management: Safe and sustainable protection of small-scale brassicas and tomatoes. Natural Resources Institute, University of Greenwich, Kent, UK.

Dong J.-H., Cheng X.-F., Yin Y.-Y., Fang Q., Ding M., Li T.-T., Zhang L.-Z., Su X.-X., McBeath J.H., Zhang Z.-K., 2008. Characterization of Tomato zonate spot virus, a new *Tospovirus* in China. *Archives of Virology* **153**: 855-864.

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**: 1203.

Elliot R.M., 2009. Bunyaviruses and climate change. *Clinical Microbiology and Infection* **15**: 510-517.

El-Wahab A.S.E-D, El-Sheik M.A-K., Elnager S., 2011. First record of *Frankliniella occidentalis* and *Impatiens necrotic spot virus* in Egypt. *Journal of Life Sciences* **5**: 690-696.

EPPO, 2004. Data sheets on quarantine pests: *Tomato spotted wilt virus*, *Impatiens necrotic spot tospovirus* and *Watermelon silver mottle virus*. *EPPO Bulletin* **34**: 271-279.

Fauquet C.M., Mayo M.A., Maniloff J., Desselberger U., Ball L.A., 2005. Virus taxonomy: Eighth report of the International Committee on Taxonomy of Viruses. Academic Press, NY, USA.

Gachu S.M., Muthomi J.W., Narla R.D., Nderitu J.H., Olubayo F.M., Wagacha J.M., 2012. Management of thrips (*Thrips tabaci*) in bulb onion by use of vegetable intercrops. *International Journal of AgriScience* **2**: 393-402.

Geerts-Dimitriadou C., Lu Y.-Y., Geertsema C., Goldbach R., Kormerlink R., 2012. Analysis of the *Tomato spotted wilt virus* ambisense S RNA-encoded hairpin structure in translation. *PLoS One* **7**: e31013.

Giliomee J.H., 1989. First record of western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) from South Africa. *Journal of the Entomological Society of Southern Africa* **52**: 179-182.

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**: 750-757.

Hafez E.E., Abdelkhalek A.A., El-Morsi A.A., El-Shahaby O.A., 2012. First report of *Iris yellow spot virus* infection of garlic and Egyptian leek in Egypt. *Plant Disease* **96**: 594.

Hassani-Mehraban A., Saaijer J., Peters D., Goldbach R., Kormelink R., 2005. A new tomato-infecting *Tospovirus* in Iran. *Phytopathology* **95**: 852-858.

Hean A.F., 1938. Kromnek disease of flowers and vegetables. *Farming in South Africa* **13**: 154-156.

Hoffmann K., Qiu W.P., Moyer J.W., 2001. Overcoming host- and pathogen-mediated resistance in tomato and tobacco maps to the M RNA of *Tomato spotted wilt virus*. *Molecular Plant-Microbe Interactions* **14**: 242-249.

Hopkins J.C.F., 1940. The tobacco "kromnek" virus in Rhodesia. *Rhodesian Agricultural Journal* **37**: 326-329.

Iwaki M., Honda Y., Hanada K., Tochihara H., Yonaha T., Hokama K., Yokoyama T., 1984. Silver mottle disease of watermelon caused by *Tomato spotted wilt virus*. *Plant Disease* **68**: 1006-1008.

Jan A.T., Azzam M., Warsi M.K., Haq Q.M.R., 2012. Technical advancement in plant virus diagnosis – an appraisal. *Archives of Phytopathology and Plant Protection* **45**: 909-921.

Jones R.A.C., 2004. Using epidemiological information to develop effective integrated virus disease management strategies. *Virus Research* **100**: 5-30.

Jones D.R., 2005. Plant viruses transmitted by thrips. *European Journal of Plant Pathology* **113**: 119-157.

Karavina C., Ibaba J.D., Gubba A., 2016a. First report of *Iris yellow spot virus* infecting onion in Zimbabwe. *Plant Disease* **100**: 235.

Karavina C., Ibaba J.D., Gubba A., 2016b. First report of *Tomato spotted wilt virus* infecting butternut squash (*Cucurbita moschata*) in Zimbabwe. *Plant Disease* **100**: 870.

Karavina C., Ibaba J.D., Gubba, A., Pappu H.R., 2016c. First report of *Iris yellow spot virus* infecting garlic and leek in Zimbabwe. *Plant Disease* **100**: 657.

Kato K., Hanada K., Kameya-Iwaki M., 2000. Melon yellow spot virus: a distinct species of the genus *Tospovirus* isolated from melon. *Phytopathology* **90**: 422-426.

Kay I.R., Herron G.A., 2010. Evaluation of existing and new insecticides including spirotetramat and pyridalyl to control *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) on peppers in Queensland. *Australian Journal of Entomology* **49**: 175-181.

King A.M.Q., Adams M.J., Carstens E.B., Lefkowitz E.J., 2012. Virus taxonomy- classification and nomenclature of viruses. Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, USA.

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.

Kouassi N.K., Wendy M., Boonham N., Smith J., 2010. Development of a diagnostic protocol for *Cucumber mosaic virus* for screening banana (*Musa* spp) planting material in Ivory Coast. *Acta Horticulture* **879**: 547-552.

Law M.D., Moyer J.W., 1990. A tomato spotted wilt-like virus with a serologically distinct N protein. *Journal of General Virology* **71**: 933-938.

Lobin K., Pappu H.R., Benimadhu S.P., 2012. Occurrence and distribution of *Iris yellow spot virus* on onion in Mauritius. Online. *Plant Health Progress* doi: 10.1094/PHP-2012-0826-01-BR.

Lokesh B., Rashmi P.R., Amruta B.S., Srisathiyanarayan D., Murthy M.R.N., Savithri H.S., 2010. NSs encoded by *Groundnut bud necrosis virus* is a bifunctional enzyme. *PLoS ONE* **5**: e9757.

Lopez C., Aramburu J., Galipienso L., Soler S., Nuez F., Rubio L., 2011. Evolutionary analysis of tomato Sw-5 resistance breaking isolates of *Tomato spotted wilt virus*. *Journal of General Virology* **92**: 210-215.

Macharia I., Backhouse D., Ateka E.M., Wu S.-B, Harvey J., Njahira M., Skilton RA., 2015a. Distribution and genetic diversity of *Tomato spotted wilt virus* following an incursion into Kenya. *Annals of Applied Biology* **166**: 520-529.

Macharia I., Backhouse D., Skilton R., Ateka E., Wu S-B., Njahira M., Maina S., Harvey J., 2015b. Diversity of thrips species and vectors of *Tomato spotted wilt virus* in tomato production systems in Kenya. *Journal of Economic Entomology* **108**: 20-28.

Macharia I., Backhouse D., Wu S.-B., Ateka E.M., 2016. Weed species in tomato production and their role as alternative hosts of *Tomato spotted wilt virus* and its vector *Frankliniella occidentalis*. *Annals of Applied Biology*. Available at: DOI 10.1111/aab.12297.

Mahy B.W.J., van Regenmortel M.H.V., 2008. Encyclopaedia of Virology. 3 Ed. Elservier Academic Press. USA.

Mandal B., Jain R.K., Krishnaredy M., Krishna Kumar N.K., Ravi K.S., Pappu H.R., 2012. Emerging problems of tospoviruses (*Bunyaviridae*) and their management in the Indian subcontinent. *Plant Disease* **96**: 468-478.

Margaria P., Ciuffo M., Pacifico D., Turina M., 2007. Evidence that the nonstructural protein of *Tomato spotted wilt virus* is the avirulence gene determinant in the interaction with resistant pepper carrying *Tsw* gene. *Molecular Plant-Microbe Interactions* **20**: 547-558.

Margaria P., Bosco L., Vallino M., Ciuffo M., Mautino G.C., Tavella L., Turina M., 2014. The NSs protein of *Tomato spotted wilt virus* is required for persistent infection and transmission by *Frankliniella occidentalis*. *Journal of Virology* **88**: 5788-5802.

Masuka A.J., Cole P.L., Mguni C., 1998. List of plant diseases in Zimbabwe. Plant Protection Research Institute, Harare, Zimbabwe.

McMichael L.A., Persley D.M., Thomas J.E., 2002. The first record of a serotype IV *Tospovirus* in Australia. *Australasian Plant Pathology* **31**: 231-239.

McMillan R.T., Graves W., 1999. *Impatiens necrotic spot tospovirus*. In: *Proceedings of the Florida State Horticultural Society* **112**: 290-291.

Meena R.L., Ramasubramanian T., Venkatesan S., Mohankumar S., 2005. Molecular characterization of *Tospovirus*-transmitting thrips populations from India. *American Journal of Biochemistry and Biotechnology* **1**: 167-172.

Melzer M.J., Tripathi S., Matsumoto T., Keith L., Sugamo J., Borth W.B., Wieczorek A., Gonsalves D., Hu J.S., 2012. Tomato Spotted Wilt. University of Hawai'i at Manoa, College of Tropical Agriculture and Human Resources. *Plant Disease* PD-81.

Moritz G., Kumm S., Mound L., 2004. *Tospovirus* transmission depends on thrips ontogeny. *Virus Research* **100**: 143-149.

Mukhopadhyay S., 2011. Plant virus, vector epidemiology and management. Science Publishers, Enfield, UK.

Ndunguru J., Taylor N.J., Yadav J., Aly H., Legg J.P., Aveling T., Thompson G., Fauquet C.M., 2005. Application of FTA technology for sampling, recovery and molecular characterization of viral pathogens and virus-derived transgenes from plant tissues. *Virology Journal* **2**: 45.

Nyamupingidza T.N., Machakaire V., 2003. Virus diseases of important vegetables in Zimbabwe. In: Hughes J.d'A., Odu J. (eds). *Plant virology in sub-Saharan Africa. Proceedings of a conference organized by The International Institute of Tropical Agriculture*, Ibadan 2001: 397-406.

Nyasani J.O., Meyhofer R., Subramanian S., Poehling H.-M., 2013. Seasonal abundance of western flower thrips and its natural enemies in different French bean agroecosystems in Kenya. *Journal of Pesticide Science* **86**: 515-523.

Nyasani J.O., Subramanian S., Poehling H.-M., Maniania N.K., Ekesi S., Meyhofer R., 2015. Optimizing western flower thrips management on French beans by combined use of beneficials and imidacloprid. *Insects* **6**: 279-296.

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**: 219-236.

Parrella G., Gognalous P., Gebre-Selassie K., Vovlas C., Marchoux G., 2003. An update on the host range of *Tomato spotted wilt virus*. *Journal of Plant Pathology* **85**: 227-264.

Perry K.L., Miller L., Williams L., 2005. *Impatiens necrotic spot virus* in greenhouse-grown potatoes in New York State. *Plant Disease* **89**: 340.

Pietersen G., Morris J., 2002. Natural occurrence of *Groundnut ringspot virus* on soybean in South Africa. *Plant Disease* **86**: 1271.

Pozzer L., Bezerra I.C., Kormelink R., Prins M., Peters D., Resende R., De O., de Avila A.C., 1999. Characterization of a *Tospovirus* isolate of *Iris yellow spot virus* associated with a disease in onion fields in Brazil. *Plant Disease* **83**: 345-350.

Predajna L., Subr Z., Adam J., Palkovics L., Glasa M., 2012. Use of FTA (Whatman) membranes for efficient and easy-to-use sampling and molecular detection of stone fruit and grapevine RNA viruses. *Acta Fytotechnica et Zootechnica*. Special Number: 44-45.

Rebijith K.B., Asokan R., Krishna V., Ranjitha H.H., Krishna Kumar N.K., Ramamurthy V.V., 2014. DNA barcoding and elucidation of cryptic diversity in thrips (Thysanoptera). *The Florida Entomologis*t **97**: 1328-1347.

Riley D.G., Shimat V.J., Srinivasan R., Diffle S., 2011. Thrips vectors of tospoviruses. *Journal of Integrated Pest Management* **1**: 1-10.

Robene-Soustrade I., Hostachy B., Roux-Cuvelier M., Minatchy J., Hedont M., Pallas R., Couteau A., Cassam N., Wuster G., 2005. First report of *Iris yellow spot virus* in onion bulb and seed production fields in Reunion Islands. *New Disease Reports* **11**: 22.

Rotenberg D., Jacobson A.L., Schneweis D.J., Whitfield A.E., 2015. Thrips transmission of tospoviruses. *Current Opinion in Virology* **15**: 80-89.

Rothwell A., 1982. A revised list of plant diseases occurring in Zimbabwe. *Kirkia* **12**: 233-352.

Sether D.M., DeAngelis J.D., 1992. *Tomato spotted wilt virus* list and bibliography. Agriculture Experimental Station, Special Report 888, p.16. Department of Entomology, Oregon State University, Corvallis, USA.

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**: 169-175.

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**: 428-434.

Soler S., Cebolla-Cornejo J., Nuez F., 2003. Control of diseases induced by tospoviruses in tomato: an update on genetic approach. *Phytopathologia Meditteranea* **42**: 207-219.

Spadotti D.M.A., Leao E.U., Rocha K.C.G., Pavan M.A., Krause-Sakate R., 2014. First report of *Groundnut ringspot virus* in cucumber fruit in Brazil. *New Disease Reports* **29**: 25.

Steward G.F., Culley A.I., 2010. Extraction and purification of nucleic acids from viruses. In Wilhelm S.W., Weinbauer M.G., Suttle C.A. (eds). Manual of aquatic virology, pp. 154-165. American Society of Limnology and Oceanography, Waco, TX, USA.

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**: 68-75.

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**: 609-625.

Tsompana M., Abad J., Purugganan M., Moyer J.W., 2005. The molecular population genetics of *Tomato spotted wilt virus* (TSWV) genome. *Molecular Ecology* **14**: 53-66.

Turina M., Tavella L., Ciuffo M., 2012. Tospoviruses in the Mediterranean area. *Advances in Virus Research* **84**: 403-437.

Uhlenhaut C., Kracht M., 2005. Viral infectivity is maintained by an RNA protection buffer. *Journal of Virology Methods* **128**: 189-191.

Wang X., Guo J.Z., 2004. *Impatiens necrotic spot virus* and its control. *Plant Quarantine* **18**: 86-87.

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

Webster C.G., Turechek W.W., Mellinger H.C., Frantz G., Roe N., Yonce H., Vallad G.E., Adkins S., 2011. Expansion of *Groundnut ringspot virus* host and geographical ranges in solanaceous vegetables in peninsular Florida. *Plant Healt Progress*. Available at DOI 10.1094/PHP-2011-0725-01-BR.

Whitfield A.E., Ullman D.E., German T.L., 2005. *Tospovirus*-thrips interaction. *Annual Review of Phytopathology* **43**: 459-489.

Winter S., Shahraeen N., Koerbler M., Lesemann D.-E., 2005. Characterization of Tomato yellow ring virus: a new *Tospovirus* species infecting tomato in Iran. *New Disease Reports* **11**: 23.

Zarzynska-Nowak A., Rymelska N., Borodynko N., Hasiow-Jaroszewska. 2016. The occurrence of Tomato yellow ring virus on tomato in Poland. *Plant Disease* **100**: 234.

Zheng K., Liu H., Yin Y., Chen T-C., Yeh S-D., Zhang Z., Dong J., 2016. Full length M and L RNA sequences of *Tospovirus* isolate 2009-GZT, which causes necrotic ringspot on tomato in China. *Archives of Virology* **161**: 1411-1414.

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