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Macharia, I., Backhouse, D., Wu, S., & Ateka, E. (2016). Weed species in tomato production and their role as alternate hosts of Tomato spotted wilt virus and its vector Frankliniella occidentalis. *Annals of Applied Biology, 169*(2), 224-235

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1 **Macharia et al. Weeds as reservoir of TSWV**

2

3 **Weed species in tomato production and their role as alternate hosts of Tomato spotted**

4 **wilt virus and its vector *Frankliniella occidentalis***

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Abstract

Tomato spotted wilt virus (TSWV) is an important plant virus that infects a wide range of hosts including weeds making its management difficult. A survey was undertaken to establish the occurrence of weed species in tomato production systems in Kenya and their role as hosts of TSWV and its vectors. Selected weed species were further evaluated for their reaction to TSWV, transmission efficiency by *Frankliniella occidentalis* and ability to support thrips reproduction. Of the 43 weed species identified in the field, 29 species had been reported as hosts of TSWV, two were non hosts and 11 had no record of their status. Among the more common species, *Amaranthus hybridus*, *Solanum nigrum*, *Tagetes minuta*, and *Datura stramonium* were susceptible to the virus and supported high levels of thrips reproduction. TSWV could not be transmitted to *Galinsoga parviflora* and *Sonchus oleraceus* by *F. occidentalis* despite them being highly susceptible in mechanical transmission tests. There was a significant correlation between feeding damage and number of larvae of *F. occidentalis* on different weeds. Occurrence of weeds that support thrips reproduction and are good hosts of TSWV is a clear indicator of their role in epidemiology and the importance of their management for disease control.

Key words: Weed species, transmission, western flower thrips, oviposition, TSWV, epidemiology

40 **Introduction**

41 *Tomato spotted wilt virus* (TSWV) (genus: *Tospovirus*; family: *Bunyaviridae*) is one of the
42 most devastating plant viruses. It infects and causes disease on many economically important
43 plant species including vegetables and ornamentals (Scholthof *et al.*, 2011; Hanssen *et al.*,
44 2010; German *et al.*, 1992). It is transmitted by at least nine species of thrips in a persistent
45 and propagative manner (Riley *et al.*, 2011). *Frankliniella occidentalis* Pergande is
46 considered to be the most important vector (German *et al.*, 1992). The virus has a unique
47 relationship with the vector where transmission occurs only when it is acquired during the
48 larval stage (Wijkamp *et al.*, 1993), with no transmission when adults are fed on infected
49 plant materials. The virus has been shown to replicate in the insect body, and infected adult
50 thrips remain viruliferous throughout their life (Whitfield *et al.*, 2005; Nagata *et al.*, 2002;
51 Kritzman *et al.*, 2002; Assis Filho *et al.*, 2002).

52 The virus infects a wide range of host plants comprising over 1090 plant species in
53 over 84 plant families (Parrella *et al.*, 2003). Its wide host range increases the difficulty of
54 managing the virus. The virus is not transmitted through seeds and there is no record of trans-
55 ovarian transmission, which indicates that each generation of thrips must acquire the virus for
56 new infection to occur (Wijkamp *et al.*, 1995; van de Wetering, 1999). Therefore, host plants
57 should support thrips vector populations for at least a generation to function as a source of
58 TSWV inoculum. In most of the East African countries tomato production is mainly practiced
59 during the dry period to avoid foliar diseases (Ssekyewa, 2006; Macharia *et al.*; Masinde *et*
60 *al.*, 2011). The virus and vector must therefore survive in alternate hosts during the tomato
61 free production period.

62 Weeds have been reported as important alternate hosts of Tospoviruses and have been
63 shown to act as reservoirs of the virus between cropping seasons (Parrella *et al.*, 2003;
64 Gracia *et al.*, 1999). 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, suitability for thrips reproduction, 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 infestation by thrips.

Several studies on the importance of weeds as reservoirs of TSWW and as hosts of different thrips species have been done 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; Okazaki *et al.*, 2007; Wilson, 1998; Groves *et al.*, 2002). Few similar studies have been done in tropical areas. Differences in environment will lead to differences in the 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.

Materials and methods

Survey of weed species in tomato production

A survey was carried out to establish 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,

90 37.5° E), and Bungoma (Bungoma county, 0.6° N, 34.6° E) in June-July 2013. Tomato farms
91 were selected randomly within the four production areas. Weeds occurring in the tomato
92 fields in each farm were sampled using five random quadrats measuring 1 m × 1m. Weed
93 species were identified, counted, and their growth stage recorded. A total of 88 farms were
94 surveyed at Kirinyaga (24), Nakuru (29), Loitokitok (14) and Bungoma (21).
95

96 **Transmission of TSWV through mechanical inoculation**

97 A greenhouse assay was done to determine the reaction of selected weed species to TSWV
98 infection. The weeds used in the study were selected based on their abundance, family, life
99 form and growth characteristics. Because the experiments were conducted in Australia, seeds
100 were collected from natural infestations in north eastern New South Wales (Table 1).
101 Collected weeds consisted of 13 common species that had been observed in Kenya and an
102 additional three weed species (*Bidens subalternans, Datura ferox* and *Solanum*
103 *chenopodioides*) resembling and easily confused with some of the weeds observed in Kenya.
104 *Commelina benghalensis* could not be obtained so *C. cyanea* was substituted in the
105 experiments. Two tomato varieties, Moneymaker as susceptible and Swanson as resistant
106 were used as controls. The seeds were pre-treated with either cold treatment or mechanical
107 scarification or both before they were established in the greenhouse in trays containing
108 potting mixture. The seedlings were transferred into 2 kg pots with soil after attaining 2-4
109 true leaves depending on weed species. After establishment, weeds were mechanically
110 inoculated with TSWV using sap from *Datura stramonium* that had been inoculated with an
111 aggressive strain of TSWV from peanut. Leaf tissue was extracted in 0.1M potassium
112 phosphate buffer, pH 7.2, containing 1% sodium sulphite and 1% carborundum powder and
113 the extract rubbed onto all but the youngest leaves of each plant. All plants were kept for 24 h
114 in the dark before and after inoculation to enhance their susceptibility to the virus.

115 Inoculation was repeated 3 times to ensure effective infection and to avoid escapes. The
116 interval between inoculations ranged from 2-5 days depending on the damage caused to the
117 plants. The weeds were maintained in an insect proof greenhouse (temperature 25±2 °C) and
118 observed for symptom expression. The treatments were replicated 10 times in a completely
119 randomized design. Data on incidence were collected at least 30 days after inoculation
120 depending on growth rate and symptom development. Leaf samples were assayed using a
121 DAS-ELISA kit (Agdia, Elkhart, IN, USA) where ELISA readings were presumed to indicate
122 virus concentration.

123 For DAS-ELISA, sap was extracted by grinding leaf samples in general extraction
124 buffer provided in the kit at a ratio of 1:10 (w/v) and 100 µl of the sample was loaded into a
125 well in a microtitre plate coated with TSWV specific antibody. Positive and negative controls
126 were also included. The plate was incubated overnight at 4°C after which the plate was
127 washed with phosphate buffered saline-Tween 20 (PBS-T) buffer. The plates were further
128 processed according to the manufacturer's protocol. Absorbance (A_{405}) was recorded after 1 h
129 incubation using a microplate reader (Epoch, BioTEK, VT, USA). Samples with absorbance
130 equal to or greater than 3 times the average negative control were considered positive.

131

132 **Laboratory culture of *F. occidentalis***

133 *F. occidentalis* was sourced from Biological Services, Loxton, South Australia through Prof.
134 Graham Hall. The identity of the thrips was confirmed using an online morphological key
135 (Mound *et al.*, 2014). The thrips were reared on cucumber using a modified rearing
136 technique based on DeGraaf and Wood (2009). Cucumbers were placed into 2 L glass jars
137 with lids covered with 150 µm mesh. The culture was maintained at 25ºC with 16:8 hours
138 light and dark. The rearing was synchronized with cucumbers being changed after every 3

139 days into new jars to allow hatching and larval development leading to different stages of
140 thrips that were used in different experiments. The jars were cleaned after every 3 weeks.
141 Viruliferous thrips were established by feeding freshly emerged first larval instars
142 with tomato leaves infected with TSWV. The larvae were allowed an acquisition period of
143 24-48 hour. The larvae were then reared to maturity and the resulting adult thrips were
144 maintained on cucumbers in glass jars.
145 Quantitative reverse transcription-PCR (qRT-PCR) was used to identify and quantify
146 TSWV in individual thrips (Rotenberg *et al*. 2009). Total RNA was extracted from individual
147 insects using the method described by Boonham *et al.* (2002) by homogenizing in nuclease
148 free water and mixing with 50µl of a 50% (w/v) slurry of Chelex 100 (Bio-Rad). The samples
149 were heated at 94°C for 5 min and centrifuged for 5 min at 13000 *g* at 4°C. The supernatant
150 was used as template in qRT-PCR. The *F. occidentalis* actin primers described by Boonham
151 *et al.* (2002) (forward primer 5′-GGT ATC GTC CTG GAC TCT GGT G-3′ and reverse
152 primer 5′-GGG AAG GGC GTA ACCT TCA-3′) were used to amplify the actin gene as an
153 internal reference and TSWV nucleocapsid (N) gene primers described by Whitfield *et al.*
154 (2008) (forward primer 5′-GCT TCC CAC CCT TT GAT TC-3′ and reverse primer 5′-ATA
155 GCC AAG ACA ACA CTG ATC-3′) were used for the virus. The one step reaction mix
156 consisted of 1-2 µl of total RNA extract, 10 µl SensiFAST™ SYBR® no-ROX one step mix
157 (Bioline, Sydney, Australia), 200 nM of each primer, 0.2 µl reverse transcriptase and 0.4 µl
158 RiboSafe RNase Inhibitor in a final volume of 20 µl. Reverse transcription was performed at
159 45° C for 10 min followed by DNA denaturation at 95° C for 2 min and 40 amplification
160 cycles of 95°C for 5 s and 60°C for 20 s in a Rotorgene 6000 real-time PCR machine
161 (Qiagen, Germany). Melting curve analysis was done after the final PCR cycle by increasing
162 the temperature by 0.5°C per min from 60°C to 95°C. The abundance of TSWV-N RNA was

163 calculated using the inverse equation of (Pfaffl, 2001): $E_{actin}^{Ct(actin)}/E_{N}^{Ct(N)}$; where E = PCR

164 efficiency of a primer pair (actin or N), and Ct = the amplification cycle.

165

166 **Transmission of TSWV using *F. occidentalis***

167 The potential of *F.occidentalis* to transmit TSWV from tomato to selected weed species was

168 evaluated. Weeds were established in the greenhouse as described above and after attaining

169 3-5 true leaves, 6 plants of each species were transferred into thrips proof cages measuring 60

170 × 60 × 60 cm (BugDorm, Taichung, Taiwan) and infested with 10 viruliferous *F. occidentalis*

171 adults per plant raised from larvae on infected tomato leaves as described earlier. Virus titre

172 in thrips was evaluated in samples collected before inoculation using qRT-PCR. Weeds were

173 maintained in the greenhouse and observed for symptom development. The feeding damage

174 from thrips infestation was evaluated on a scale of 0-3 (Maharijaya *et al.*, 2011) while the

175 reproduction potential was evaluated qualitatively by tapping the plants over a white surface

176 and observing the relative abundance of larvae. The plants were assayed for TSWV infection

177 three weeks after infestation using DAS ELISA kits (Agdia, USA) and the infection was

178 confirmed using reverse transcription PCR (RT-PCR).

179 RNA was extracted from weed samples using Trizol reagent (Life Technologies,

180 Mulgrave, VIC Australia) according to the manufacturer's protocol. Leaf tissue (100 mg) was

181 ground to fine powder in liquid nitrogen and 1 mL of Trizol reagent was added to each tube

182 and vortexed thoroughly and incubated for 5 min at room temperature to allow complete

183 dissociation of nucleoprotein complexes. Chloroform was added into the mixture at the rate

184 of 200 µl per 1ml of Trizol used, shaken vigorously and incubated for 3-5 minutes at room

185 temperature. The tube was centrifuged at 13000 *g* for 15 min at 4°C and the upper aqueous

186 phase transferred into a fresh 1.5 ml sterile tube. RNA was precipitated by adding 500 µl of

187 ice-cold isopropanol and the tube incubated for 10 min at room temperature. The tubes were

188 centrifuged at 13000 *g* for 10 min. The supernatant was discarded and the pellet washed with
189 500 µl of 75% (v/v) ethanol. After drying, the pellet was resuspended in 50 µl of RNase-free
190 water and stored at -80°C.

191 Reverse transcription PCR (RT-PCR) was performed using two primer sets: TSWV
192 722 (5'-GCT GGA GCT AAG TAT AGC AGC-3') and TSWV 723 (5'-CAC AAG GCA
193 AAG ACC TTG AG-3') (Adkins and Rosskopf, 2002); and TSW 1 (5'-TCT GGT AGC ATT
194 CAA CTT CAA-3') and TSW 2 (5'-GTT TCA CTG TAA TGT TCC ATA G-3') (Roberts *et*
195 *al.*, 2000). One step RT-PCR was carried out using the QIAGEN OneStep RT-PCR Kit
196 (Qiagen, Chadstone, VIC, Australia) with 1 µl of RNA extract in a 25 µl reaction mix of 5 µl
197 of 5× one step PCR buffer (containing 2.5mM $MgCl_2$), 400 µM dNTP mix, 0.4 µM of
198 forward and reverse primers and 1 µl of one step RT-PCR enzyme mix (containing reverse
199 transcriptase and Hotstar Taq DNA polymerase). The reactions for TSW 1 and 2 were
200 performed as follows: reverse transcription at 48°C for 45 min; 95°C for 15 min to activate
201 the Hotstar Taq DNA polymerase and inactivate reverse transcriptase; 40 cycles of 94°C for
202 45 s denaturation of cDNA, 45°C for 45 s annealing and 72°C for 1 min extension; and a
203 final extension at 72°C for 10 min. The reactions for TSWV 722 and 723 were similar except
204 that annealing was done at 55 °C for 45 s, and 35 cycles of amplification performed. Three µl
205 of resulting PCR products were mixed with 6 µl of orange G loading buffer (150 mg/mL
206 Ficoll 400, 2.5 mg/mL Orange G) containing 1:200 (v:v) GelStar™ Nucleic Acid Gel Stain
207 (Lonza, Rockland, ME, USA) and visualized in a 1.5% agarose gel. The expected sizes were
208 620bp (TSWV 722 and 723) and 628bp (TSW1 and 2).

209
210 **Evaluation of thrips reproduction on detached leaves**

211 Selected weed species and the tomato varieties Grosse Lisse and Moneymaker (TSWV-
212 susceptible) and Klass and Swanson (TSWV-resistant) were evaluated for their ability to
213 support reproduction of *F. occidentalis* using a detached leaf assay. The detached leaves were

214 placed in a 9 cm diameter Petri dish with a window in the lid covered with 150 µm mesh
215 containing 1% water agar amended with 0.01% Terraclor fungicide (750 mg/g
216 pentachloronitrobenzene) to prevent fungus growth. Five adult thrips were introduced onto
217 each leaf and the Petri dish sealed with Parafilm to prevent the thrips from escaping. Thrips
218 were allowed to feed and oviposit for 3 d under 16:8h light/dark, at temperature of 25ºC and
219 50-60% relative humidity. After 3 d, adult thrips were removed and number of larvae and
220 feeding punctures/damage was recorded. The feeding or damage was assessed using a
221 relative scale from 0 (no damage) to 3 (severe damage) (Maharijaya *et al.*, 2011). The Petri
222 dishes were re-sealed with Parafilm and incubated as above for an additional 3 days. The
223 larvae that emerged from each leaf in each weed species were counted.
224

225 **RESULTS**

226 **Survey of weed species in tomato production**

227 A total of 43 species of weeds representing 19 families were found in the tomato production
228 areas (Table 2). The family Asteraceae had the highest (9) number of species while
229 Solanaceae and Poaceae had six and five species, respectively (Table 2). Most of the weeds
230 (29 species) have previously been recorded as hosts of TSWV, while two species (*Ricinus*
231 *communis* and *Digitaria scalarum*) have been reported as non-hosts (Table 2). Eleven
232 species, including the relatively abundant *Commelina benghalensis* and *Oxalis latifolia*, were
233 of unknown host status. The majority of the weed species identified were annual (27 species),
234 12 were perennial and the remainder could be either annual or short lived perennial (Table 2).
235 *Bidens pilosa, Amaranthus hybridus, Galinsoga parviflora,* and *Commelina*
236 *benghalensis* were the most frequently found weed species across the four production areas
237 (Table 3). The highest number of weed species (34) was found in the Kirinyaga area while 25
238 species were found at each of Nakuru and Bungoma and 23 species at Loitokitok. Among the

239 observed weeds, 21 occurred in at least 3 of the production areas while 16 occurred in only
240 one of the production areas. Most of these were found at low frequency, except
241 *Acanthospermum hispidum* and *Richardia brasiliensis* which were found on 58% and 54%
242 respectively of the farms at Kirinyaga (Table 3).
243
244 **Transmission of TSWV through mechanical inoculation**
245 Twelve out of the 17 weed species that were evaluated became infected with TSWV after
246 mechanical inoculation (Table 4). Infected weeds produced a wide range of characteristic
247 symptoms with mosaic and stunting being the most common symptoms. Some of the plants
248 that were positive in the ELISA test did not produce any symptoms. This was true for all
249 infected plants of *Malva parviflora*, but also for individual plants of several other species.
250 *Chenopodium album* produced necrotic local spots and the virus was only identified from the
251 inoculated leaves. Absorbance in the ELISA test for these leaves was just above the threshold
252 for a positive result. Incidence varied among the weed species with the highest disease
253 incidence being recorded on *Datura stramonium, D. ferox,* and *C. album*. *Amaranthus*
254 *hybridus and G. parviflora* had the highest virus titre equivalent recorded. All weeds in the
255 Solanaceae family had high virus incidence and a high virus titre ranging from 2.8 to 3.2
256 which was comparable with the susceptible tomato Moneymaker (3.0) used as control (Table
257 4). No infections were observed on *Bidens pilosa, Trifolium repens*, *Commelina cyanea, O.*
258 *latifolia*, *Tagetes minuta* or the resistant tomato Swanson after mechanical inoculation.
259
260 **Transmission of TSWV using *F. occidentalis***
261 Potentially viruliferous *F. occidentalis* successfully transmitted TSWV from infected tomato
262 leaves to many of the weed species (Table 5). Quantitative RT-PCR confirmed that *F.*
263 *occidentalis* had acquired the virus. Relative TSWV titre in individual thrips samples ranged

264 from a normalized value of 0.15 to 283.4 relative to actin gene expression, while the

265 normalized value for the healthy control was 0.0015. The rate of acquisition of TSWV by *F.*

266 *occidentalis* was 45% of 75 individuals tested.

267 *Bidens pilosa, C. cyanea, C. album, D. stramonium, B. subalternans, T. repens and T.*

268 *minuta* produced characteristic symptoms ranging from mosaic, necrotic local lesions,

269 necrotic spots, vein clearing, stunting and leaf distortion (Table 5). Some of the other infected

270 weed species did not produce any symptoms. TSWV infection of weeds was identified using

271 DAS-ELISA and RT-PCR tests (Table 5). The highest incidence of infection was observed

272 on *A. hybridus*, *O. latifolia*, *S. chenopodioides, C. album* and *C. cyanea* while no infection

273 was detected on *G. parviflora* and *Sonchus oleraceus*. The experiment was repeated for these

274 species, again with negative results.

275 *A. hydridus, B. pilosa, G. parviflora, M. parviflora, S. chenopodioides, T. repens and*

276 *T. minuta* supported high survival and reproduction of thrips based on the number of larvae

277 recovered after the experiment (Table 5). Weed species that supported high survival and

278 reproduction of thrips also showed high levels of feeding damage.

279

280 **Evaluation of thrips reproduction on detached leaves**

281 Selected weed species were found to be suitable feeding hosts and supported reproduction of

282 *F occidentalis*. Feeding damage was significantly different (Kruskal-Wallis test $\chi^2 = 102.2$, df

283 $= 20$, $P < 0.001$) among weed species. The highest damage was observed on *A. hybridus* (2.5)

284 and lowest damage was observed on *P. oleracea* (0.3) (Figure 1a). The TSWV-susceptible

285 tomato varieties Grosse Lisse and Moneymaker showed less damage than the resistant

286 varieties Klass and Swanson. Levels of feeding damage in the detached leaf assay (Figure 1a)

287 were consistent with those observed in the greenhouse experiment (Table 5).

288 Reproduction of *F. occidentalis* was significantly different between weed species

289 (Kruskal-Wallis $\chi^2 = 116.68$, df = 20, $P < 0.001$). Highest numbers of larvae were seen on *B.*

290 *subalternans* while *S. oleraceus, C. album*, whereas *P. oleracea* had the lowest. Plants with

291 high larval counts were ranked from *B. subalternans* > *T. minuta* > *S. nigrum* > *A. hybridus* >

292 *B. pilosa* > *G. parviflora* > *M. parviflora* ≥ Grosse Lisse > *D. stramonium* = Moneymaker

293 (Fig. 1b). TSWV-susceptible tomato cultivars supported more thrips larvae than the resistant

294 cultivars. There was a significant positive correlation ($r = 0.679$, $P < 0.001$) between feeding

295 damage and number of larvae indicating that thrips oviposited more eggs on hosts they

296 perceived as suitable. Numbers of larvae counted in the detached leaf assay (Figure 1b) were

297 consistent with qualitative assessments of larval numbers in the greenhouse experiment

298 (Table 5).

299

300 **Discussion**

301 Our results revealed occurrence of a wide range of weeds in major tomato production areas in

302 Kenya. The weeds represented 19 plant families. Of these Asteraceae and Solanaceae had the

303 highest number of species recorded and were distributed in all the production areas. Most of

304 the weeds species that had previously been identified as hosts of the virus were also in the

305 family Asteraceae followed by Solanaceae which is consistent with observations that these

306 families contain large numbers of plants susceptible to TSWV (Parrella *et al.*, 2003). The

307 large number of weeds observed indicates the presence of suitable hosts able to support the

308 virus and its vectors if introduced.

309 The transmission study resulted in four new hosts of TSWV, *O. latifolia, Bidens*

310 *subalternans, S. chenopodioides* and *C. cyanea*. The first three species belong to genera with

311 other known hosts. However, no plants in the family Commelinaceae have yet been recorded

312 as susceptible to TSWV. Although *C. cyanea* did not occur in the survey area, it is likely that

313 the closely related species *C. benghalensis* would show similar susceptibility to TSWV as *C.*
314 *cyanea*.
315 Transmission of TSWV to weeds depends both on their susceptibility to the virus and
316 their suitability as feeding and oviposition hosts of the vector (Kahn *et al.*, 2005). Of the
317 weeds chosen for detailed study, all had at least one plant infected by either mechanical or
318 thrips transmission. Although TSWV was not mechanically transmitted onto *B. pilosa, T.*
319 *repens, O. latifolia, C. cyanea,* or *T. minuta*, there was successful transmission through the
320 vector, *F. occidentalis.* In some cases, such as in *O. latifolia*, infection was only detected by
321 RT-PCR so the apparent failure of mechanical transmission may have been because DAS-
322 ELISA, which was the only detection method used in the mechanical transmission
323 experiment, was particularly insensitive in this species. Other species may have had
324 anatomical or chemical characteristics that interfered with virus transmission when the plant
325 surface was damaged by carborundum. More interesting are cases where mechanical
326 inoculation was successful, showing susceptibility to the virus, but where infection could not
327 be established by thrips transmission.
328 The ability of the weeds to support *F. occidentalis* feeding and reproduction was
329 assessed using a detached leaf assay under laboratory condition and qualitative data from the
330 greenhouse experiment. Thrips laid high numbers of eggs on plants that they perceived as
331 suitable feeding host evidenced by the high correlation between feeding damage and number
332 of larvae. Thrips have been reported to distinguish the suitability of plants as feeding and
333 oviposition hosts to ensure fitness of their progeny (Scott Brown *et al.*, 2002; Nyasani *et al.*,
334 2013). The reproduction potential recorded in the detached leaf assay was similar to the
335 greenhouse data. The oviposition and reproductive potential are influenced by the nutritional
336 quality of host plant and presence or absence of plant defense compounds (Delphia *et al.*,
337 2007; Shrestha *et al.*, 2012).

338 *Amaranthus hybridus, D. stramonium and S. nigrum* had high frequency in the field
339 and supported high thrips reproduction. Although these species had high transmission rates
340 by mechanical inoculation, there was varied vector transmission with the highest transmission
341 recorded in *A. hydridus*. *Datura stramonium* was found to be a good host for TSWV
342 acquisition by *F. occidentalis* (Bautista *et al.*, 1995), as it had a high virus titre and an even
343 distribution of infected cells. Furthermore, *D. stramonium* was reported as a good TSWV
344 acquisition and transmission host of *T. tabaci* (Chatzivassiliou *et al.*, 2007; Chatzivassiliou *et*
345 *al.*, 1999). This reinforces the significance of *D. stramonium* as observed in our current study.
346 However, *A. hybridus,* an annual weed that proliferates fast with several generations per year.
347 This could allow it to support rapid increases in populations of thrips.
348 *Bidens pilosa*, *G. parviflora* and *S. oleraceus* occurred frequently across the four
349 production areas. All three species have frequently been cited as hosts of TSWV but there is
350 conflicting evidence for their role in transmission of the virus by *F. occidentalis*.
351 Transmission of TSWV to these species by thrips was either very low (*B. pilosa*) or
352 undetectable (*G. parviflora* and *S. oleraceus)* in our experiments, although *G. parviflora* and
353 *S. oleraceus* were readily infected by mechanical transmission. (Chatzivassiliou *et al.*, 2001)
354 found a high incidence of TSWV infection in *S. oleraceus* in the field in Greece. However,
355 (Chatzivassiliou *et al.*, 2007) were unable to transmit TSWV to *S. oleraceus* using *T. tabaci*
356 due to high larval mortality and poor oviposition preference. We also found the species to be
357 a poor feeding and oviposition host for *F. occidentalis.* Presumably *S. oleraceus* can become
358 infected when there is a large population of viruliferous thrips that has built up on other
359 species, but it is unlikely to be attractive to thrips if other plants are available nearby.
360 On the other hand, *B. pilosa* and *G. parviflora* supported a high rate of thrips
361 reproduction but had a low level of TSWV acquisition. There have been reports of high
362 frequency of TSWV infection in field populations of *B. pilosa*, but incidence of infection in

363 *G. parviflora* was low (Cho *et al.*, 1986). Because these are common weeds in many cropping
364 systems, there is need for further work to establish their importance as reservoirs of the virus.
365 *Portulaca oleracea* was shown to be susceptible to TSWV but supported low feeding
366 and reproduction by thrips. (Atakan *et al.*, 2013) found a high incidence of TSWV infection
367 in *P. oleracea* in the field in Turkey, but reported that this species did not favour reproduction
368 of *F. occidentalis*. However, this species and *S. oleraceus* may be less important as reservoirs
369 of the virus than their abundance suggests if they only support low populations of larvae.
370 Further work is needed to compare transmission from infected weed plants to economically
371 important hosts like tomato.
372 *Oxalis latifolia* and *C. benghalensis* are perennial weed species identified as hosts of
373 TSWV. *Oxalis latifolia* occurred frequently at Bungoma and Nakuru while *Commelina*
374 *benghalensis* was common in all areas. Although these weeds supported relatively low thrips
375 reproduction, their status as hosts of TSWV makes them play an important role as their
376 perennial nature enables them to persist in the field for a long time and withstand harsh
377 environmental conditions. Perennial weed species serves as persistent source of virus
378 inoculum which is later passed onto annual weeds where it replicates before it is further
379 spread to susceptible crops (Persley *et al.*, 2006; Wilson, 1998). This indicates that these
380 weeds should not be ignored while developing management options for TSWV in tomato
381 production.
382 *Malva parviflora* was an interesting weed species as it consistently supported high
383 thrips reproduction, feeding damage and TSWV infection. Although no symptoms were
384 observed on the infected plants analysis of the asymptomatic plants through DAS-ELISA and
385 RT-PCR indicated they were positive for TSWV. This finding is consistent with observations
386 made by (Bautista *et al.*, 1995),who found a high incidence of asymptomatic TSWV infection
387 and a strong attraction of *F. occidentalis* to *M. parviflora*. As a long-lived annual or perennial

388 weed *M. parviflora* has the ability to remain longer in the field making it an important silent
389 reservoir of TSWV.

390 In conclusion the study has revealed occurrence of numerous weed species in major
391 tomato production areas most of which can support TSWV and are hosts of *F. occidentalis*.
392 Some of the weed species had special attributes which enhance their importance in TSWV
393 epidemiology. This supports the importance of weed management as a component in the
394 integrated management of TSWV and its vectors. However there is need to further evaluate
395 the reproduction potential and persistence of these weeds to better understand their
396 contribution with the view of developing effective and cost effective management options.
397

398 **Acknowledgments**

399 The authors wish to appreciate the support from AusAID for an Australian Development
400 Scholarship to I. M. and for supporting the research activity. We also wish to acknowledge
401 Dennis Persley of the Queensland Department of Agriculture, Fisheries and Forestry for
402 TSWV isolates, Prof. Graham Hall for facilitating acquisition of the *Frankliniella*
403 *occidentalis* culture and Matthew Binns for confirming identity of the *F. occidentalis* used in
404 the study.
405

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544
545
546

547 **Table 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

548 [1]Relative abundance of the species in surveys in Kenya.

549 [2]Species not recorded during survey. *Commelina benghalensis* had high abundance.

550

551

552 **Table 2.** Weeds identified in tomato production areas in Kenya, biological type and their
553 status as hosts of TSWV

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)

554

555 **Table 3**. Frequency (%) of occurrence of weed species in four tomato production areas in
556 Kenya. Species are ranked in order of overall frequency.
557

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	-	-

558
559

560 **Table 4.** Symptoms, incidence and absorbance in the ELISA test of selected weed species
561 mechanically inoculated with TSWV
562

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	-

563 [a] **NR**-Necrotic ringspot, **NS**-Necrotic spot, **M**-Mosaic, **Mo-**Mottle, **LD**-leaf deformation, **ST**-
564 stunting, **P**- Purpling, **C**-Chlorosis, (-) no symptom
565 [b] Number of plants positive by ELISA over number of plants tested.
566 [c] ELISA average is based on the absorbance for the positive samples. Absorbance of negative
567 controls and plants recorded as negative was ≤ 0.1.

568 **Table 5.** Transmission of Tomato spotted wilt virus by *F. occidentalis* from tomatoes to selected weed species

569

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	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	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	M	H	–	0/6	0/6	0/6
Galinsoga parviflora[f]	2	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	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

570

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

572 [c] Symptoms: M = Mosaic, VC = Vein clearing, NL = Necrotic lesion, NS = Necrotic spots, P = Purpling, ST = Stunting, LD = Leaf deformation, –

573 = No symptom

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

575 [f] Result of experiments that were repeated.

576 Figure legends

577 **Fig 1a**. Level of damage from *F. occidentalis* infestation on weed species and tomato

578 cultivars in a detached leaf assay.

579 **Fig 1b**. Average number of larvae of *F. occidentalis* on weed species and tomato cultivars in

580 a detached leaf assay.

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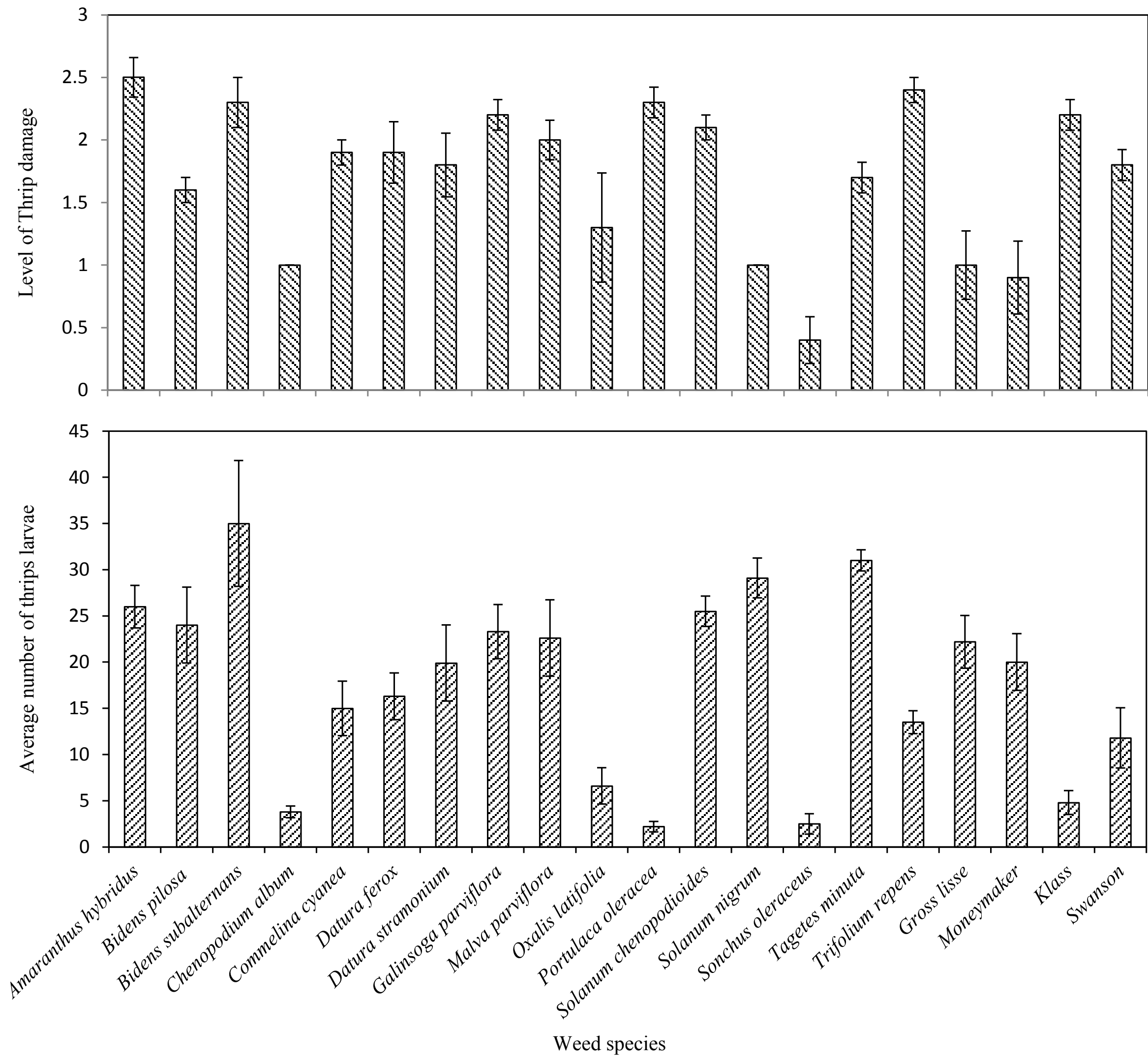

584

585 Figure 1a & b

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