

1 MAIZE CHLOROTIC MOTTLE VIRUS INDUCES CHANGES IN HOST PLANT VOLATILES THAT
2 ATTRACT VECTOR THRIPS SPECIES
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12
13 **Abstract** - Maize lethal necrosis is one of the most devastating diseases of maize causing yield losses reaching up to
14 90% in sub-Saharan Africa. The disease is caused by a combination of maize chlorotic mottle virus (MCMV) and
15 any one of cereal viruses in the Potyviridae group such as sugarcane mosaic virus. MCMV has been reported to be
16 transmitted mainly by maize thrips (*Frankliniella williamsi*) and onion thrips (*Thrips tabaci*). To better understand
17 the role of thrips vectors in the epidemiology of the disease, we investigated behavioral responses of *F. williamsi*
18 and *T. tabaci*, to volatiles collected from maize seedlings infected with MCMV in a four-arm olfactometer bioassay.
19 Volatile profiles from MCMV-infected and healthy maize plants were compared by gas chromatography (GC) and
20 GC coupled mass spectrometry analyses. In the bioassays, both sexes of *F. williamsi* and male *T. tabaci* were
21 significantly attracted to volatiles from maize plants infected with MCMV compared to healthy plants and solvent
22 controls. Moreover, volatile analysis revealed strong induction of (E)-4,8-dimethyl-1,3,7-nonatriene, methyl
23 salicylate and (E,E)-4,8,12-trimethyltrideca-1,3,7,11-tetraene in MCMV-infected maize seedlings. Our findings
24 demonstrate MCMV induces changes in volatile profiles of host plants to elicit attraction of thrips vectors. The
25 increased vector contact rates with MCMV-infected host plants could enhance virus transmission if thrips feed on
26 the infected plants and acquire the pathogen prior to dispersal. Uncovering the mechanisms mediating interactions
27 between vectors, host plants and pathogens provides useful insights for understanding the vector ecology and
28 disease epidemiology, which in turn may contribute in designing integrated vector management strategies. **Key**
29 **Words** - Multi-trophic interactions, maize chlorotic mottle virus, *Frankliniella williamsi*, *Thrips tabaci*, behavioral
30 assays, induced volatile compounds.

INTRODUCTION

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Maize, *Zea mays* L. (Poaceae), is a major staple and cash crop for over 300 million people in sub-Saharan Africa (SSA), covering a production area of over 27 M ha and is mainly grown by smallholder farmers (Sileshi et al. 2010; Cairns et al. 2013). Unfortunately, maize production in SSA is constrained by several indigenous and invasive pests and diseases resulting in significant yield loss (Kifr et al. 2002). The maize lethal necrosis (MLN) disease syndrome recently reported in eastern and central Africa on maize and other cereal crops (Wangai et al. 2012; Adams et al. 2014; Kusia et al. 2015; Mahuku et al. 2015b; Isabirye and Rwomushana 2016) is among the most important viral diseases that can cause up to 90% yield loss and has a devastating impact on food security and livelihoods (Mahuku et al. 2015a). The best strategy to manage the disease is to employ integrated pest management practices encompassing different approaches such as vector control and host-plant resistance (Nelson et al. 2011; Wangai et al. 2012). The disease occurs due to co-infection of cereal crops, such as maize and finger millets, with *Maize chlorotic mottle virus* (MCMV) and *Sugarcane mosaic virus* (SCMV) (Wangai et al. 2012; Kusia et al. 2015).

The maize chlorotic mottle virus (Tombusviridae: Machlomovirus), one of the causative agents of MLN, has been spreading rapidly to various locations around the world in the past decade (Braidwood et al. 2018; Mahuku et al. 2015a). MCMV single infection in maize leads to yield losses that range between 10 to 15% in natural infection, whereas up to 59% yield loss has been reported in artificially inoculated maize plots (Uyemoto, 1983). More importantly, MCMV interaction with other Potyviridae viruses such as sugarcane mosaic virus causes an aggressive synergistic viral condition - maize lethal necrosis, which often leads to complete crop losses (Braidwood et al. 2018; Mahuku et al. 2015a; Wang et al. 2017; Xie et al. 2011). Recently, rapid spread of such synergistic viral infection has occurred in Africa, China, Taiwan and Ecuador where it has led to severe necrosis and yield losses in maize, sweet corn and finger millets (Degen et al. 2014; Kusia et al. 2015; Mahuku et al. 2015a; Wang et al. 2017; Xie et al. 2011). In Africa, the first MCMV outbreak was reported from the southern rift valley of Kenya in 2011 (Wangai et al. 2012). MCMV and consequently MLN incidences in eastern and central Africa have been on increase since its first incidence (Adams et al. 2014; Lukanda et al. 2014; Mahuku et al. 2015b). The potential spread of MCMV to maize producing regions across Africa and its synergistic interaction with established potyviruses to cause MLN has been predicted (Isabirye and Rwomushana 2016). MCMV is transmitted mechanically in a semi-persistent manner by several vectors including, maize thrips (*Frankliniella williamsi*) (Cabanas et al. 2013), Chrysomelid beetles (*Oulemamelanopa*) and corn rootworms (*Diabrotica* spp) (Nelson et al. 2011). In eastern Africa, thrips have been observed in high densities in fields affected by MLN and MCMV (Mahuku et al. 2015a; Wangai et al. 2012). Maize thrips (*F. williamsi*), onion thrips (*Thrips tabaci*) and the pale form of common blossom thrips (*Frankliniella schultzei*) are known to transmit MCMV in east Africa and are widely distributed in the region (Mahuku et al. 2015a; Nyasani et al. 2015). *Frankliniella williamsi* and *T. tabaci* have narrow host range of Poaceae, Amaryllidaceae and Brassicaceae, while *F. schultzei* has a much wider host range (Moritz et al. 2013). The attraction of thrips and other insect vectors to virus infected or intact plants has been proposed to be mediated by volatile organic compounds (VOCs) and/or visual cues such as leaf color, which play a crucial role in both pre- and

68 post-alighting stages of host selection (Abdullah et al. 2015; Koschier et al. 2000, 2007). VOCs emitted from virus-
69 infected plants may differ from healthy plants and this could influence the preference of vectors such as thrips for
70 infected plants (Abe et al. 2011; De Vos and Jander 2010). Several studies have revealed that plants infected by
71 pathogens are attractive to insect vectors of the pathogens and support better survival and development of vectors
72 than uninfected plants though the mechanisms underpinning the interactions have not been adequately examined
73 (Belluire et al. 2005; Eigenbrode et al. 2002; Tomitaka et al. 2015).

74
75 Plant viruses are known to manipulate their vectors' behavior via host plant nutrients and volatiles to enhance their
76 transmission and spread (Blanc and Michalakis 2016; Mauck et al. 2014; Shalileh et al. 2016; Tomitaka et al. 2015).
77 Information on virus-thrips-host plant interactions is available to some extent for thrips – tospovirus interactions
78 (Abe et al. 2011; Tomitaka et al. 2015), where the mode of virus transmission is persistent circulative (Whitfield et
79 al. 2005). However, there is a scarcity of information on mechanisms mediating multitrophic interactions between
80 host plant–thrips–MCMV, where the mode of virus transmission is semi-persistent and noncirculative (Cabanas et
81 al. 2013). Hence, this study was designed to examine the chemical ecology of MCMV-vectorhost plant interactions.
82 The behavioural responses of two thrips species, i.e. maize thrips (*F. williamsi*) and onion thrips (*T. tabaci*), to
83 maize volatiles inoculated with MCMV and healthy maize plants were investigated. Moreover, the volatile profiles
84 from MCMV infected and healthy maize plants were characterized and compared by gas chromatography (GC) and
85 GC coupled mass spectrometry (GC-MS). Information on the underlying mechanism mediating thrips vectors,
86 MCMV and maize plants interactions may help in better understanding of vector ecology and epidemiology of the
87 pathogen.

88

METHODS AND MATERIALS

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90 *Plants and Virus Inoculation.* Disease-free maize seeds of variety H6210 were planted singly in pots (21cm in
91 height, 20cm diameter) filled with sterilized (autoclaved) soil in an insect-proof screen house at the International
92 Centre of Insect Physiology and Ecology (*icipe*), Duduville campus, Nairobi. Three weeks-old plants, at principal
93 phenological growth stage one (BBCH-scale, Lancashire et al. 1991) were used in experiments. The plants were
94 artificially inoculated with *Maize chlorotic mottle virus* inoculum consisting of infected leaf sap in 0.01 M
95 potassium phosphate buffer (PH 7.0) and carborundum 100 mesh grit (Wangai et al. 2012). Application to the host
96 plant was done using a soft finger-rubbing technique, i.e. by dipping cheesecloth-tied fingers in the inoculums, and
97 gently rubbing the maize plant leaves and later incubating in a separate screen house for one week before use in the
98 experiments. Concurrently, control plants were treated the same way, but without virus inoculum.

99

100 *Insects.* Adult maize thrips, *Frankliniella williamsi* and onion thrips, *Thrips tabaci* were obtained from thrips
101 cultures maintained at the Thrips IPM program lab at *icipe*, Duduville campus. The thrips culture was originally
102 initiated through adult thrips that were field-collected from maize and onions fields in the Central Kenya. The field-
103 collected insects were reared on baby corn, *Zea mays* and snow peas, *Pisum sativum*, respectively as described by
104 Nyasani et al. (2013) and maintained in ventilated plastic jars (17 cm in height, 8 cm diameter) at 25±1°C, 50–60%
105 relative humidity (RH) and 12L: 12D photoperiod. The laboratory-reared adult thrips used in various behavioral
106 assays were maintained for more than 30 generations in the lab with intermittent infusion of field collected thrips to
107 keep the original behavioral characteristics of the species. Identification and separation of male and female adult
108 thrips was based on visible external morphological features under a stereomicroscope (Moritz et al. 2013). Female
109 and male thrips of each species were aspirated and transferred separately into ventilated plastic jars.

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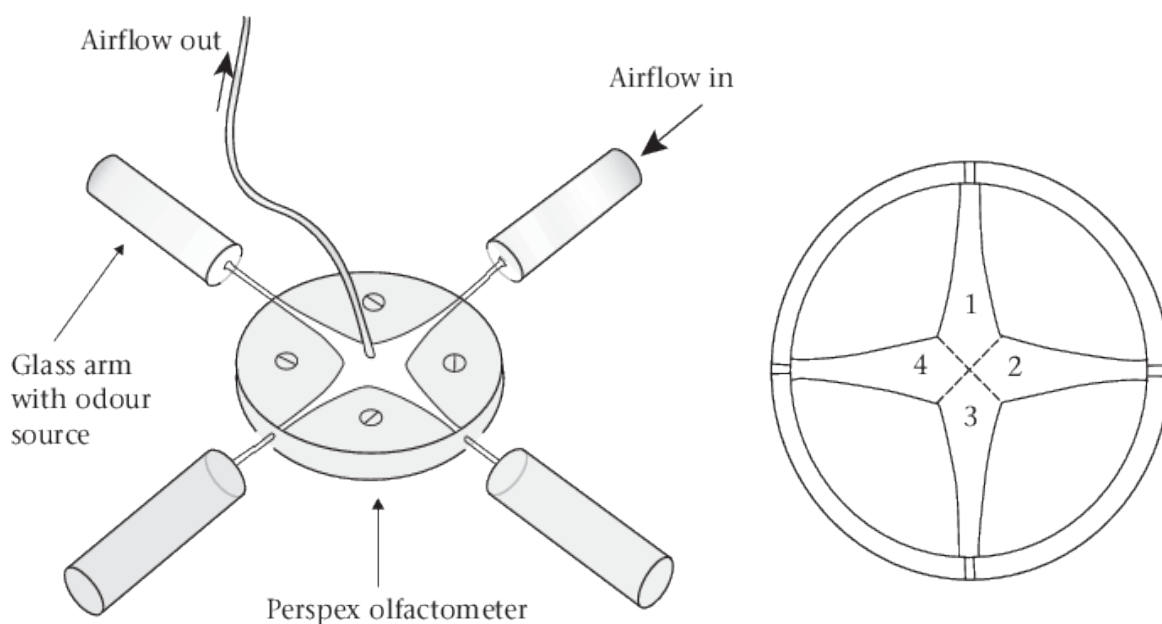
111 *Collection of Plant Volatiles.* Plant volatiles from maize seedlings infected with MCMV ($N=6$) and healthy plants
112 ($N=6$) were collected by headspace sampling (Tamiru et al. 2011). Prior to volatile collection, individual maize
113 plants were placed inside odourless polyethyleneterephthalate (PET) bags (volume 3.2L, ~12.5 mm thickness)
114 heated to 100°C for 1 hour before use and fitted with Swagelock inlet and outlet ports. The bottom of each bag was
115 tightened around the plant stem and the upper bag opening closed with a twist-on seal. Charcoal-filtered air was
116 pumped constantly at 500 ml min⁻¹ through the inlet port for 24 hrs. Headspace volatiles were simultaneously
117 collected at room temperature on Porapak Q (0.05 g, 60/80 mesh; Supelco) filters inserted in the outlet port through
118 which air were drawn at 300 ml min⁻¹. After entrainment, volatiles retained in the Porapak Q filters were desorbed
119 with 0.5 ml dichloromethane. Each sample was stored at -20°C in individual small glass vials (2 ml, Agilent
120 Technologies) with polytetrafluoroethylene (PTFE) lined screw cap until used in the bioassay and GC and GC-MS
121 analyses.

122

123 *Four-arm Olfactometer Bioassay.* Behavioural responses of the two thrips species to volatiles from MCMV infected
124 and healthy maize seedlings were tested in a Perspex four-arm olfactometer as described in Tamiru et al. (2011)
125 (Figure 1). Headspace samples (10µl aliquots) were applied, using a micropipette (Drummond ‘microcap’,

126 Drummond Scientific Co., Broomall, PA, USA), to pieces of filter papers (4×25 mm) placed in the inlet port at the
127 end of each olfactometer arm. A choice-test was carried out where the two opposite arms held the test stimuli (10µl
128 aliquots of headspace sample) and the remaining two arms were solvent controls. Putative non-viruliferous male and
129 female thrips of each species were starved for 24 hrs and acclimatized at a room temperature for 2 hrs. A single
130 adult thrip of specific species and sex was individually transferred into the central chamber of the olfactometer using
131 a soft camel-hair brush. Air was drawn through the four open-ended olfactometer arms towards the centre at 260 ml
132 min⁻¹. The time spent by thrips in each olfactometer arm was recorded with 'Olfa' software (F. Nazzi, Udine, Italy)
133 for 12 min. To avoid directional bias, the position of the treatments was randomly allocated between each replicate
134 and the olfactometer arms were gently rotated 90° after every 3 min during the test. Each olfactometer was used only
135 once per replicate and scrupulously cleaned before the next bioassay run. The olfactometers were washed with an
136 aqueous solution of Teepol, 80% ethanol and rinsed with distilled water and air-dried; whereas, the glass arms were
137 further cleaned with acetone and sterilized in an oven at 150°C for 2 hrs. The experiment was replicated 12 times
138 (each insect representing a replicate). Test insects were discarded when they remained motionless for more than 2
139 uninterrupted minutes and replaced with new ones.

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143 **FIG. 1** A schematic diagram representing the four-arm olfactometer (120 mm diameter) with cylindrical glass arms
144 (90 mm × 20 mm internal diameter with 50 mm × 3mm internal diameter connecting arms) used to contain odour
145 sources alongside diagram showing division of regions within the olfactometer.

146

147 *Gas Chromatographic (GC) Analysis.* GC analysis was carried out by injecting 2 µl of headspace sample onto an
148 Agilent 6890 GC equipped with a cross-linked methyl silicone capillary column (HP-1, 50 m, 0.32 mm i.d, 0.52 µm
149 film thickness) fitted with a cool-on-column injector and a flame ionization detector (FID). The carrier gas was
150 hydrogen. The oven was maintained at 30 °C for 1 min and then programmed at 5 °C min⁻¹ to 150 °C and 10 °C min⁻¹

151 ¹ to 250 °C with a total run time of 55.1 min. Quantification of the volatile compounds was performed with a
152 multiple point external standard calibration method using GC traces peak area data acquired from nine different
153 concentrations (0.001, 0.005, 0.01, 0.05, 1, 5, 10, 25, 50 ng µl⁻¹) of authentic standards.

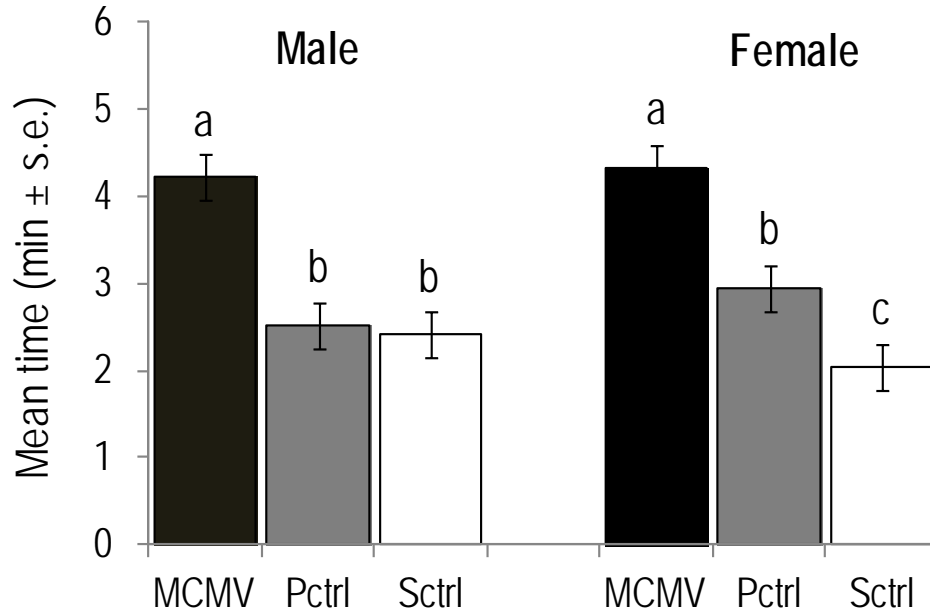
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155 *Coupled GC-Mass Spectrometry (GC-MS) Analysis.* Aliquots of headspace volatile samples (1µl) from MCMV
156 infected and healthy plants were analysed with VG AutoSpec mass spectrometer (Fisons Instruments, Manchester,
157 UK) coupled to a Hewlett Packard 5890 GC equipped with a cool-on-column injector. Ionization was performed by
158 electron impact (70 eV, 220°C). To separate the volatiles, non-polar column (HP-1, 50 m, 0.32 mm i. d., 0.52 µm
159 film thickness) was used with Helium as carrier gas at constant flow. The oven temperature was maintained at 30°C
160 for 1 min, then programmed at 5°C min⁻¹ to 150 °C and 10 °C min⁻¹ to 250 °C with a total run time of 70 min. The
161 volatiles were then identified by comparison of retention times and mass spectra with those of authentic standards,
162 reference library (NIST05) and with MS data published in the literature. Identifications were confirmed by peak
163 enhancement with authentic samples (Tamiru et al. 2011).

164
165 *Data Analyses.* Statistical analyses were performed using R statistical software, version 3.2.3 (R Core Team 2015).
166 Time spent in each arm of the four-arm olfactometer bioassay was compared by analysis of variance (ANOVA) after
167 conversion of the data into proportions and a log ratio transformation. Significant means were separated using
168 Student Newman Keul (SNK) test. All tests were performed at 5% significance level.

170 RESULTS

171
172 *Behavioral Responses of Frankliniella williamsi:* Both female and male *F. williamsi* spent significantly more time in
173 the olfactometer arm containing volatiles from plants inoculated with MCMV in comparison to those containing
174 volatiles from healthy plants and solvent controls (Male: $F=7.67$, $P=0.0014$; Female: $F=13.52$, $P<0.0001$) (Figure
175 2). The time spent by males in areas with volatiles from MCMV infected plants was 1.69 and 1.78 times higher than
176 in areas with volatiles from uninfected plants and solvent control, respectively. On the other hand, females spent
177 1.47 and 2.02 times more time in areas with volatiles from MCMV infected plants compared to areas with volatiles
178 from uninfected plants and solvent control, respectively.

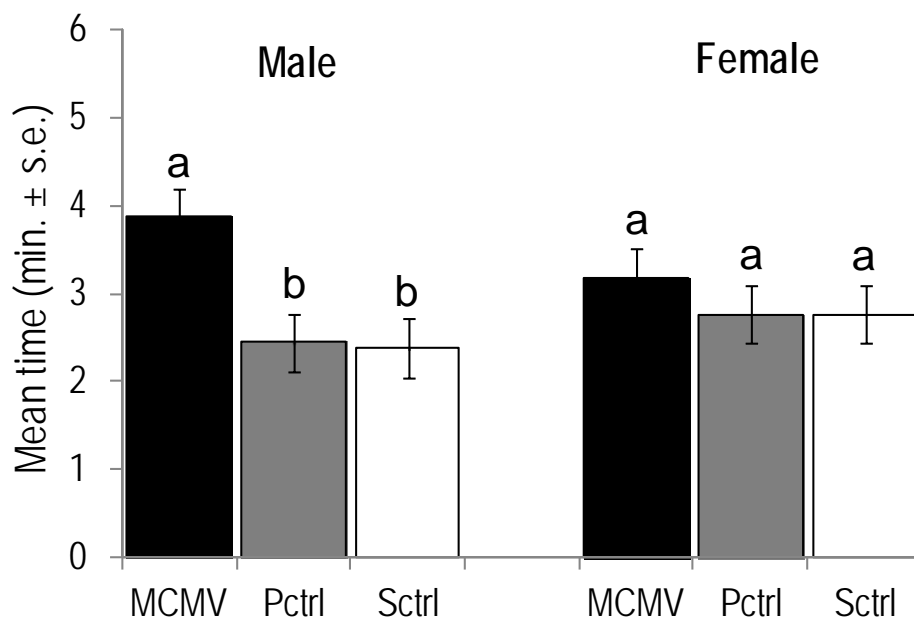
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 181 FIG. 2 Behavioral responses of maize thrips, *Frankliniella williamsi*, to maize volatiles from *Maize chlorotic mottle*
 182 *virus* (MCMV) infected plants, healthy plants (Pctrl) and solvent control (Sctrl) in a four-arm olfactometer bioassay.
 183 Each insect was observed for 12 min ($N = 12$). Mean time spent $\pm SE$ (minutes) by *F. williamsi* in each part of the
 184 olfactometer is shown. Different letters above the bars indicate statistically significant differences based on the
 185 Student–Newman–Keuls (SNK) test ($P < 0.05$).

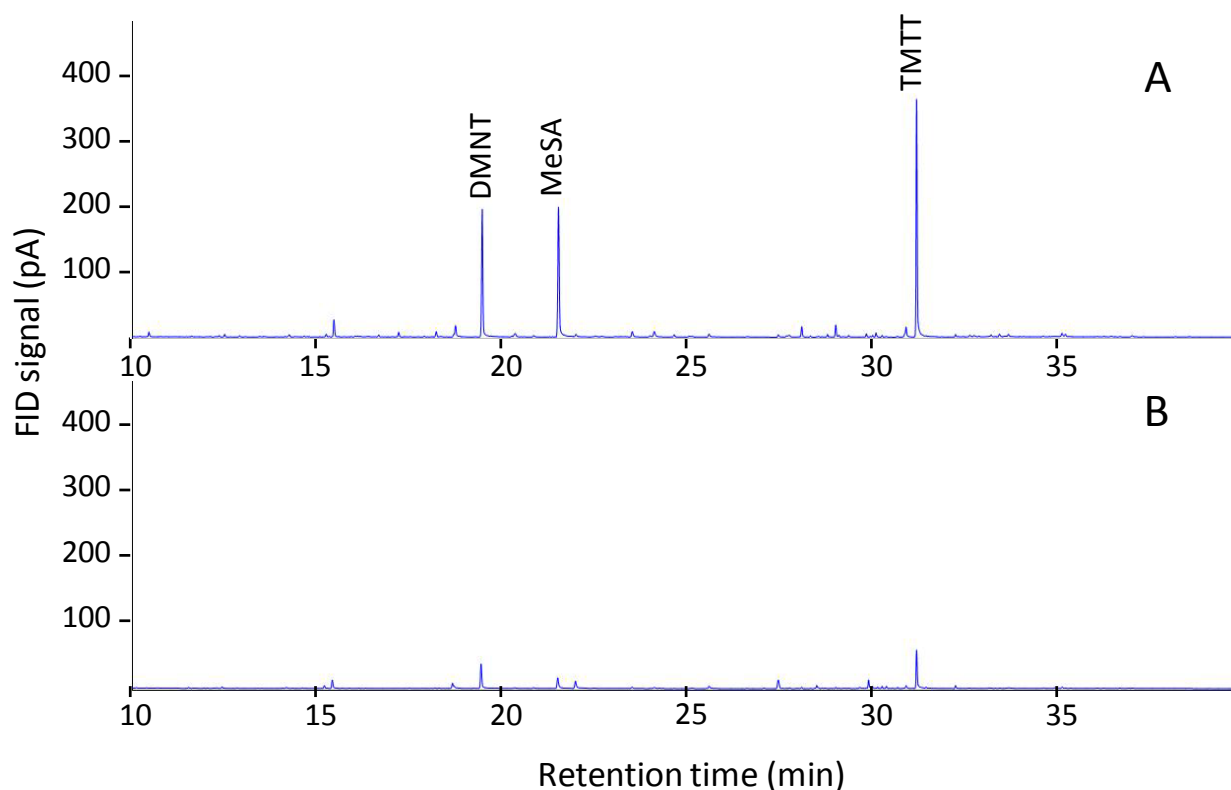
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 187 *Behavioral Responses of Thrips tabaci.* Male *T. tabaci* were significantly attracted to volatiles from MCMV
 188 inoculated plants compared to healthy plants and solvent controls ($F=3.98$, $P=0.027$) (Figure 3). The male
 189 preference for volatile for MCMV infected plants was 1.58 and 1.62 times higher than for volatiles from healthy
 190 plants and solvent control, respectively. However, there was no significant difference between time spent by females
 191 in olfactometer arms containing volatiles from MCMV inoculated plant and non-inoculated plants and solvent
 192 controls ($F=0.79$, $P=0.4590$) (Figure 3).

193



194
 195 **FIG. 3** Behavioral responses of onion thrips, *Thrips tabaci*, to maize volatiles from *Maize chlorotic mottle virus*
 196 (MCMV) inoculated plants, healthy plants (Pctrl) and solvent controls (Sctrl) in a four-arm olfactometer bioassay.
 197 Each insect was observed for 12 min ($N = 12$). Time spent (min; mean \pm SE) by *T. tabaci* in each part of the
 198 olfactometer is shown. Different letters above the bars indicate statistically significant differences based on the
 199 Student–Newman–Keuls (SNK) test ($P < 0.05$).

200
 201 *Volatile Analysis.* Chemical analysis of headspace samples revealed qualitative and quantitative changes in the
 202 volatile profiles of MCMV infected and uninfected (healthy) maize plants (Figure 4). There was strong induction of
 203 (*E*)-4, 8-dimethyl-1,3,7-nonatriene (DMNT), methyl salicylate (MeSA) and (*E,E*)-4,8,12-trimethyltrideca-1,3,7,11-
 204 tetraene (TMTT) on maize plants inoculated with MCMV compared to healthy plants (Figure 4). MCMV inoculated
 205 maize plants emitted significantly higher amounts of the bioactive compounds DMNT, MeSA and TMTT compared
 206 to healthy plants. Mean emission rates (ng kg^{-1} fresh weight hr^{-1}) of the major volatile compounds in MCMV
 207 infected and healthy plants are presented in Table 1.



209
 210 FIG. 4 GC profiles of headspace volatiles from (A) *Maize chlorotic mottle virus* infected and (B) healthy maize
 211 seedlings. There was strong induction of (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), methyl salicylate (MeSA) and
 212 (*E,E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT) in maize plants infected with MCMV. The X-axis
 213 represents retention time in minutes (min) while Y-axis gas chromatography–flame ionization detector (GC–FID)
 214 signal in pico-ampere (pA).

215
 216 TABLE 1. Emission rates of strongly induced volatiles compounds from maize plants inoculated with the *Maize*
 217 *chlorotic mottle virus* (MCMV) and healthy plants

Volatiles compounds	Mean volatile emission rates (ng kg fresh weight ⁻¹ hr ⁻¹) (±SE)		F	P
	MCMV infected Plants	Healthy Plants		
(<i>E</i>)-4,8-dimethyl-1,3,7-nonatriene (DMNT)	18.70 (±1.09) a*	4.13 (±1.34) b	59.28	0.0046
Methyl salicylate (MeSA)	58.29 (±9.35) a	4.20 (±1.45) b	79.25	0.0030
(<i>E,E</i>)-4,8,12-trimethyltrideca- 1,3,7,11-tetraene (TMTT)	26.84 (±4.33) a	6.33 (±1.73) b	27.54	0.0135

218 *Means followed by different letters, within a row, are significantly different (N=6, SNK test $P < 0.05$)

219

DISCUSSION

220
221 Our results revealed that infection of maize plants with the maize chlorotic mottle virus (MCMV) induces changes
222 in volatile profiles of plants leading to significant attraction of thrips vectors *F. williamsi*, and *T. tabaci* to the
223 infected plants. There was a strong induction of volatile compounds (E)-4,8- dimethyl-1,3,7-nonatriene (DMNT),
224 methyl salicylate (MeSA), (E,E)-4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT) on maize seedlings inoculated
225 with MCMV. The significant increase in the release of these compounds corresponded with the attractiveness of the
226 headspace samples from MCMV inoculated plants to the thrips vectors. Previous studies have shown that plant
227 viruses induce changes in plant volatile profiles which, in turn, may affect the behavioral responses of their vectors
228 (Eigenbrode et al. 2002; Mauck et al. 2014; Oluwafemi et al. 2011). For example, the level of MeSA has been
229 shown to increase dramatically on tobacco (*Nicotiana tabacum*) after inoculation with the tobacco mosaic virus
230 (Seskar et al. 1998). Similarly, high levels of bioactive compounds such as linalool and DMNT were observed in
231 cucumber mosaic virus infected chilli plants (*Capsicum annuum*) and on tomato plants infected with tomato spotted
232 wilt virus (Maris 2004; Saad et al. 2017). However, there is paucity of information about induction of changes in the
233 host plant volatile profiles due to MCMV infection or other viruses belonging to the family Tombusviridae. Results
234 from our study demonstrated induction of changes in volatile profiles of maize plants due to MCMV infection which
235 elicited positive behavioral responses in the thrips vectors. We have also characterized the key volatile
236 semiochemicals mediating thrips vectors-MCMV-host plant interactions. Understanding the underlying mechanism
237 mediating thrips vectors, MCMV and maize plants interactions will help to better understand vector ecology and
238 epidemiology of the MCMV. This, in turn, may provide useful inputs towards designing semiochemical based
239 integrated vector management strategy (Cook et al. 2007; Mfuti et al. 2017; Niassy et al. 2012).

240
241 In the behavioral study, both sexes of *F. williamsi* and male *T. tabaci* were significantly attracted to maize volatiles
242 infected with MCMV compared to healthy plants. This concurs with previous studies on other thrips and insect
243 species which showed preference of insect vectors for plants infected with virus (Mauck et al. 2014; Tomitaka et al.
244 2015). Host plant location by insects involves detection of specific compounds and/or blends of volatile
245 semiochemicals in specific ratios (Bruce and Pickett 2011; Tamiru et al. 2015). Studies have shown that DMNT,
246 MeSA and TMTT are produced in higher quantities after insect infestation on plants and elicit potent attraction of
247 pests' natural enemies individually and/or as a blend (de Boer and Dicke 2004; Mallinger et al. 2011; Tamiru et al.
248 2011, 2015; Turlings et al. 1998). In western flower thrips, *F. occidentalis*, DMNT and MeSA have been shown to
249 elicit behavioral responses (Chermenskaya et al. 2001; Koschier et al. 2007; Maris 2004). The strong induction of
250 the bioactive compounds DMNT, MeSA and TMTT on MCMV inoculated plants and preference of both thrips
251 species to virus induced volatiles from infected plants compared to volatiles from healthy plants suggests that
252 MCMV alters emission of maize volatiles to enhance attraction of thrips vectors. If the thrips vectors subsequently
253 feed on the infected plants for sufficient time to acquire the pathogen prior to dispersal, this attractive phenotype
254 may lead to enhanced virus spread. Adult thrips transmit MCMV for up to 6 days after acquisition with no need for
255 latent periods (Cabanas et al. 2013). Increasing number of evidences suggested the advantages of vector attraction to

256 virus infected plants in promoting disease transmission and spread (Belliere et al. 2005; Eigenbrode et al. 2002;
257 Shapiro et al. 2012; Sisterson, 2008).

258
259 Interestingly, differences in behavioral responses between male and female *T. tabaci* were observed in this study.
260 Unlike the males, female *T. tabaci* did not show preference to volatiles from MCMV infected plants. The low female
261 *T. tabaci* response to MCMV infected maize plants compared to *F. williamsi* could be attributed to the fact that
262 maize is not the primary host for *T. tabaci* although the pest is polyphagous (Moritz et al. 2013). Moreover, *T. tabaci*
263 was reared on a different host plant, i.e. snow peas, which could influence the choices that the insect makes when
264 experiencing new odor. Silva et al. (2013) reported context dependent behavioral responses of two congeneric
265 thrips, *Frankliniella schultzei* (Trybom) and *F. occidentalis* (Pergrande), to induced plants volatiles. Furthermore,
266 feeding behavior of thrips vectors to virus infected plants is known to differ between the sexes and viruliferous
267 nature of the pests. For example, males of western flower thrips, *F. occidentalis*, transmit tomato spotted wilt virus
268 more efficiently than females (Stafford et al. 2011; Van De Wetering et al. 1999). This could be due to more robust
269 virus infection of males and sexually dimorphic feeding behaviors (Rotenberg et al. 2009; Van De Wetering et al.
270 1999). Our present study provides additional evidence on sexually dimorphic behavioral responses of onion thrips,
271 *T. tabaci*, towards MCMV infect maize plants.

272 Transmission efficiency of a virus in the field is determined, among other factors, by the number of viruliferous
273 vectors in the population and their sex ratio (VanDeWetering et al. 1999), their interaction with the host plant at
274 different phases of infection (Blua and Perring, 1992) and spatial distribution of infected plants (McElhany et al.
275 1995). Hence, a better understanding on the chemical ecology of thrips vectors, MCMV and host plants interactions
276 could provide valuable insight into developing environmentally sustainable and effective thrips vectors monitoring
277 and management tools by exploiting plant derived volatile semiochemicals mediating the interactions. This, in turn,
278 will greatly contribute towards disease management efforts by mitigating virus transmission and spread while
279 maintaining ecological integrity. For example, studies have shown that addition of semiochemical attractants to
280 monitoring tools like sticky cards increases capture of thrips species such as western flower thrips and onion thrips
281 (Abdullah et al. 2015; Koschier 2008; Teulon et al. 2014). Similarly, semiochemical-baited autoinoculation devices
282 treated with fungal-based biopesticides e.g. *Metarhizium anisopliae* have been used to control thrips (Niassy et al.
283 2012). Evaluating and improving the efficacy of such control strategies through the addition of optimally attractive
284 semiochemicals into the pest's monitoring and management tools is one of the goals for improving IPM. Multi-
285 trophic interactions between insect vectors, host plants and viruses causing MLN is a complex pathosystem. Our
286 current findings established that MCMV, one of the causative agents of MLN, infection induces changes in volatile
287 profiles of maize plants to attract thrips vectors and characterized the main volatile compounds mediating the
288 interactions. Examining the role of individual volatile components and their blends in influencing thrips behavior
289 and vector competence including their effects on insects settling, virus acquisition and dispersal under laboratory
290 and field condition is an important goal for future research. This will provide further clarity on the Maize-MCMV-
291 thrips interactions and virus epidemiology and aid in designing integrated thrips and MCMV management strategies.

292 Exploiting plant volatile semiochemicals has been shown to present novel and ecologically sustainable pest
293 management opportunities (Tamiru and Khan 2017; Teulon et al. 2014).

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301 COMPLIANCE WITH ETHICAL STANDARDS

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303 *Conflict of Interest* - The authors declare that they have no conflict of interest.

304 *Ethical Approval* - Experiments were performed in accordance with relevant guidelines and regulations on studies
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307 REFERENCES

308

309 ABDULLAH, Z.S., GREENFIELD, B.P.J., FICKEN, K.J., TAYLOR, J.W.D., WOOD, M., and BUTT, T.M. 2015.

310 A new attractant for monitoring western flower thrips, *Frankliniella occidentalis* in protected crops.
311 *Springer Plus* 4:89.

312 Abe, H., Tomitaka, Y., Shimoda, T., Seo, S., Sakurai, T., Kugimiya, S., Tsuda, S. and Kobayashi, M.,
313 2011. Antagonistic plant defense system regulated by phytohormones assists interactions among
314 vector insect, thrips and a tospovirus. *Plant and Cell Physiology*, 53(1), pp.204-212.

315 ADAMS, I.P., HARJU, V.A., HODGES, T., HANY, U., SKELTON, A., RAI, S., DEKA, M.K., SMITH, J., FOX,
316 A., UZAYISENGA, B., and NGABOYISONGA, C. 2014. First report of maize lethal necrosis disease in
317 Rwanda. *New Dis. Rep.* 29:22.

318 BELLIURE B, JANSSEN A, MARIS PC, PETERS D, SABELOS MW, 2005. Herbivore arthropods benefit from
319 vectoring plantviruses. *Ecol. Lett.* 8, 70–79.

320 BLANC, S. AND MICHALAKIS, Y. 2016. Manipulation of hosts and vectors by plant viruses and impact of the
321 environment. *Current Opinion in Insect Science*, 16:36–43

322 BRUCE, T.J.A. 2010. Tackling the threat to food security caused by crop pests in the new millennium. *Food*
323 *Security*, 2: 133–141.

324 BRUCE, T.J.A. and PICKETT, J.A. 2011. Perception of plant volatile blends by herbivorous insects – finding the
325 right mix. *Phytochemistry* 72:1605–1611.

326 BRUNNER, P.C. and FREY, J.E. 2010. Habitat-specific population structure in native western flower thrips
327 *Frankliniella occidentalis* (Insecta, Thysanoptera). *J. Evol. Biol.* 23:797–804.

328 CABANAS, D., WATANABE, S., HIGASHI, C.H.V., and BRESSAN, A. 2013. Dissecting the Mode of Maize
329 Chlorotic Mottle Virus Transmission (Tombusviridae: Machlomovirus) by *Frankliniella williamsi*
330 (Thysanoptera: Thripidae). *Econ. Entomol.* 106:16–24.

331 CAIRNS, J.E., HELLIN, J., SONDER, K., ARAUS, J.L., MACROBERT, J.F., THIERFELDER, C., and
332 PRASANNA, B.M. 2013. Adapting maize production to climate change in sub-Saharan Africa. *Food*
333 *Security* 5:345–360.

334 Chermenskaya, T. D., Burov, V. N., Maniar, S. P., Pow, E. M., Roditakis, N., Selytskaya, O. G., ... &
335 Woodcock, C. M. (2001). Behavioural responses of western flower thrips, *Frankliniella*
336 *occidentalis* (Pergande), to volatiles from three aromatic plants. *International Journal of Tropical*
337 *Insect Science*, 21(1), 67-72.

338 COOK, S.M., KHAN, Z.R., AND PICKETT, J.A. 2007. The use of push-pull strategies in integrated pest
339 management. *Annu. Rev. Entomol.* 52:375–400.

340 DE BOER, J.G. and DICKE, M. 2004. The role of methyl salicylate in prey searching behavior of the predatory mite
341 *Phytoseiulus persimilis*. *J. Chem. Ecol.* 30:255–271.

342 DE VOS, M. and JANDER, G. 2010. Volatile communication in plant-aphid interactions. *Curr. Opin. Plant Biol.*
343 13:366–371.

344 EIGENBRODE, S.D., DING, H., SHIEL, P., and BERGER, P.H. 2002. Volatiles from potato plants infected with
345 *potato leafroll virus* attract and arrest the virus vector, *Myzus persicae* (Homoptera: *Aphididae*). *Proc. R.*
346 *Soc. B.* 269:455–460.

347 GAO, Y.L., LEI, Z.R., and REITZ, S.R. 2012. Western flower thrips resistance to insecticides: detection,
348 mechanisms and management strategies. *Pest Manag. Sci.* 68:1111–1121.

349 ISABIRYE, B.E. and RWOMUSHANA, I. 2016. Current and future potential distribution of *Maize chlorotic mottle*
350 *virus* and risk of maize lethal necrosis disease in Africa. *J. Crop Prot.* 5:215–228.

351 KOSCHIER, E.H., DE KOGEL, W.J., and VISSER, J.H. 2000. Assessing the attractiveness of volatile plant
352 compounds to western flower thrips *Frankliniella occidentalis*. *J. Chem. Ecol.* 26:2643–2655.

353 Koschier, E. H., Hoffmann, D., & Riefler, J. (2007). Influence of salicylaldehyde and methyl salicylate on
354 post-landing behaviour of *Frankliniella occidentalis* Pergande. *Journal of applied*
355 *entomology*, 131(5), 362-367.

356 KUSIA, E.S., SUBRAMANIAN, S., NYASANI, J.O., KHAMIS F., VILLINGER, J., ATEKA, E., and PAPPU,
357 H.R. 2015. First report of lethal necrosis disease associated with co-infection of finger millet with Maize
358 chlorotic mottle virus and Sugarcane mosaic virus in Kenya. *Plant Dis.* 99:899–900.

359 LANCASHIRE, P.D., BLEIHOLDER, H., LANGELÜDDECKE, P., STAUSS, R., VAN DEN BOOM, T.,
360 WEBER, E., and WITZENBERGER, A. 1991. A uniform decimal code for growth stages of crops and
361 weeds. *Ann. Appl. Biol.* 119:561–601.

362 MAHUKU, G., LOCKHART, B.E., WANJALA, B., JONES, M.W., KIMUNYE, J.N., STEWART, L.R.,
363 CASSONE, B.J., SEVGAN, S., NYASANI, J.O., KUSIA, E., KUMAR, P.L., NIBLETT, C.L.,
364 KIGGUNDU, A., ASEA, G., PAPPU, H.R., WANGAI, A., PRASANNA, B.M., and REDINBAUGH,

365 M.G. 2015A. Maize Lethal Necrosis (MLN), an Emerging Threat to Maize-Based Food Security in Sub-
366 Saharan Africa. *Phytopathology* 105:956–965.

367 MAHUKU, G., WANGAI, A., SADESSA, K., TEKLEWOLD, A., WEGARY, D., AYALNEH, D., ADAMS, I.,
368 SMITH, J., BOTTOMLY, E., BRYCE, S., BRAIDWOOD, L., FEYISSA, B., REGASSA, B., WANJALA,
369 B., KIMUNYE, J.N., MUGAMBI, C., MONJERO, K., and PRASANNA, B.M. 2015B. First report of
370 maize chlorotic mottle virus and maize lethal necrosis on maize in Ethiopia. *Plant Dis.* 99:1870.

371 MALLINGER, R.E., HOGG, D.B., and GRATTON, C. 2011. Methyl salicylate attracts natural enemies and reduces
372 populations of soybean aphids (Hemiptera: aphididae) in soybean agroecosystems. *J. Econ. Entomol.*
373 104:115–124.

374 Maris, P. C. 2004. Evaluation of thrips resistance in pepper to control Tomato spotted wilt virus
375 infection. PhD thesis, Wageningen University, Wageningen.

376 MAUCK, K.E., DE MORAES, C.M., and MESCHER, M.C. 2014. Biochemical and physiological mechanisms
377 underlying effects of Cucumber mosaic virus on host-plant traits that mediate transmission by aphid
378 vectors. *Plant Cell Environ.* 37:1427–1439.

379 MCELHANY, P., REAL, L.A., and POWER, A.G. 1995. Vector preference and disease dynamics: A study of
380 barley yellow dwarf virus. *Ecology* 76:444–457.

381 MFUTI, D. K., NIASSY, S., SUBRAMANIAN, S., DU PLESSIS, H., EKESI, S., and MANIANIA, N. K. 2017.
382 Lure and infect strategy for application of entomopathogenic fungus for the control of bean flower thrips in
383 cowpea. *Biol. Contr.* 107: 70-76.

384 MORITZ, G., BRANDT, S., TRIAPITSYN, S., and SUBRAMANIAN, S. 2013. Identification and Information
385 Tools for Pest Thrips in East Africa. QBIT, QAAFI, UQ. ISBN 978-1-74272-067-8
386 [http://thripsnet.zoologie.unihalle.de/key-server-neu/data/03030c05-030b-4107-880b-0a0a0702060d/media/](http://thripsnet.zoologie.unihalle.de/key-server-neu/data/03030c05-030b-4107-880b-0a0a0702060d/media/Html/index.html)
387 [Html/index.html](http://thripsnet.zoologie.unihalle.de/key-server-neu/data/03030c05-030b-4107-880b-0a0a0702060d/media/Html/index.html).

388 NDERITU, J.H., WAMBUA, E.M., OLUBAYO, F., KASINA, J.M., and WATURU, C.N. 2007. Management of
389 thrips (Thysanoptera: Thripidae) infestation on French beans (*Phaseolus vulgaris* L.) in Kenya by
390 combination of insecticides and varietal resistance. *J. Entomol.* 4:469–473.

391 NELSON S., BREWBAKER J., and HU J. 2011. Maize chlorotic mottle. Honolulu (HI): University of Hawaii. 6 p.
392 (Plant Disease; PD-79). <http://hdl.handle.net/10125/32440>

393 NYASANI, J., KUSIA, E., and SUBRAMANIAN, S. 2015. Thrips as pests and vectors of *Maize chlorotic mottle*
394 *virus* in maize. In Proc. Xth International Symposium on Thysanoptera and Tospoviruses, Asilomar
395 Conference Grounds, May 16th – 20th, 2015. P. 49.

396 R DEVELOPMENT CORE TEAM 2015. R: A Language and Environment for Statistical Computing. R Foundation
397 for Statistical Computing, Vienna, Austria.

398 ROTENBERG, D., KUMAR, N.K.K., ULLMAN, D.E., MONTERO-ASTÚA, M., WILLIS, D.K., GERMAN, T.L.,
399 and WHITFIELD, A.E. 2009. Variation in tomato spotted wilt virus titer in *Frankliniella occidentalis* and
400 its association with frequency of transmission. *Phytopathology* 99:404–410.

401 SESKAR, M., SHULAEV, V., and RASKIN, I. 1998. Endogenous Methyl Salicylate in Pathogen-Inoculated
402 Tobacco Plants. *Plant Physiol.* 116:387–392.

403 SHALILEH, S., OGADA, P.A., MOUALEU, D.P., and POEHLING, H.M. 2016. Manipulation of *Frankliniella*
404 *occidentalis* (Thysanoptera: Thripidae) by *Tomato Spotted Wilt Virus* (Tospovirus) Via the Host Plant
405 Nutrients to Enhance Its Transmission and Spread. *Environ. Entomol.* 45:1235–1242.

406 SILESHI, G., AKINNIFESI, F.K., DEBUSHO, L.K., BEEDY, T., AJAYI, O.C., and MONG'OMBA, S. 2010.
407 Variation in maize yield gaps with plant nutrient inputs, soil type and climate across sub-Saharan Africa.
408 *Field Crops Res.* 116:1–13.

409 SISTERSON, M.S. 2008. Effects of insect-vector preference for healthy or infected plants on pathogen spread,
410 insights from a model. *J. Econ Entomol.* 101:1–8.

411 STAFFORD, C.A., WALKER, G.P., and ULLMAN, D.E. 2011. Infection with a plant virus modifies vector feeding
412 behavior. *Proc. Natl. Acad. Sci. U.S.A* 108:9350–9355.

413 TAMIRU, A., BRUCE, T.J.A., WOODCOCK, C.M., BIRKETT, M.A., MIDEGA, C.A.O., PICKETT, J.A., and
414 KHAN, Z.R. 2015. Chemical cues modulating electrophysiological and behavioral responses in the
415 parasitic wasp *Cotesia sesamiae*. *Can. J. Zool.* 93:281–287.

416 TAMIRU, A., BRUCE, T.J.A., WOODCOCK, C.M., CAULIFIELD, C.J., MIDEGA, C.A.O., OGOL, C.K.P.O.,
417 MAYON, P., BIRKETT, M.A., PICKETT, J.A., and KHAN, Z.R. 2011. Maize landraces recruit egg and
418 larval parasitoids in response to egg deposition by a herbivore. *Ecol. Lett.* 14:1075–1083.

419 TAMIRU, A. and KHAN, Z.R. 2017. Volatile Semiochemical Mediated Plant Defense in Cereals: A Novel Strategy
420 for Crop Protection. *Agronomy* 7 (3): 58.

421 TEULON DAJ, CASTAÑÉ C, NIELSEN M-C, EL-SAYED AM, DAVIDSON MM, GARDNER-GEE R,
422 POULTON, KEAN, A.M, HALL, C., BUTLER, R.C., SANSOM, C.E., SUCKLING, D.M., AND PERRY
423 N.B. 2014. Evaluation of new volatile compounds as lures for western flower thrips and onion thrips in
424 New Zealand and Spain. *N Z Plant Prot* 67:175–183.

425 TOMITAKA, Y., ABE, H., SAKURAI, T., and TSUDA, S. 2015. Preference of the vector thrips *Frankliniella*
426 *occidentalis* for plants infected with thrips-non-transmissible Tomato spotted wilt virus. *J.Appl. Entomol.*
427 139: 250–259.

428 TURLINGS, T.C.J., BERNASCONI, M., BERTOSSA, R., BIGLER, F., CALOZ, G., and DORN, S. 1998. The
429 induction of volatile emissions in maize by three herbivore species with different feeding habits: possible
430 consequences for their natural enemies. *Biol. Control* 11:122–129

431 VAN DE WETERING, F., VAN DER HOEK, M., GOLDBACH, R., and PETERS, D. 1999. Differences in *Tomato*
432 *spotted wilt virus* vector competency between males and females of *Frankliniella occidentalis*. *Entomol.*
433 *Exp. Appl.* 93:105–112.

434 WANGAI, A.W., REDINBAUGH, M.G., KINYUA, Z.M., MIANO, D.W., LELEY, P.K., KASINA, M.,
435 MAHUKU, G., SCHEETS, K., and JEFFERS, D. 2012. First Report of Maize chlorotic mottle virus and
436 Maize Lethal Necrosis in Kenya. *Plant Dis.* 96:1582–1583.

437 Whitfield, A. E., Ullman, D. E., & German, T. L. (2005). Tospovirus-thrips interactions. *Annu. Rev.*
438 *Phytopathol.*, 43, 459-489.