

1 **Quantitative Phenotyping of Vascular Damage Caused by Fusarium Wilt**
2 **Disease in Cowpea**

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6 **ABSTRACT**

7 Assessment of the severity of Fusarium wilt disease in cowpea and other crops
8 relies mainly on visual rating scales which are prone to errors, which can
9 compromise the reproducibility of the data. Furthermore, the rating scales require
10 considerable practical training and routine experience for reliable assessment. Two
11 objective metrics, stem vascular discoloration length (%VDL) and number of
12 Fusarium necrotic vessels (NFNV), for quantitative measurement of vascular
13 damage incited by *Fusarium oxysporum* f. sp. *tracheiphilum* race 4 (Fot4) of cowpea,
14 were compared and their utility as a measure of disease severity and potential
15 usefulness in other crop pathosystems is proposed. The metrics were tested in
16 seven F₂ populations and one F_{2:3} population, segregating for wilt response, and
17 inoculated with race Fot4 at the seedling stage. %VDL and NFNV were highly
18 correlated with plant wilting for all populations ($r = 0.51 - 0.93$ and $0.52 - 0.94$,
19 respectively). Furthermore, the relationships between the variables were linear in all
20 populations ($R^2 = 0.81$ to 0.87 and 0.71 to 0.91), indicating that they can provide
21 accurate and reliable measurement of severity of Fusarium wilt disease. Also, %VDL
22 and NFNV were strongly correlated ($r = 0.88 - 0.97$) and demonstrated a linear
23 relationship ($R^2 = 0.69 - 0.94$). Analysis of goodness-of-fit in two F₂ populations
24 revealed that errors in measurement of vascular discoloration length can result in

25 higher segregation distortion when compared to enumeration of necrotic vessels.
26 However, both metrics were highly effective in accounting for the severity of vascular
27 damage caused by Fusarium wilt disease.

28

29 **Key words:** *Fusarium oxysporum* f. sp. *tracheiphilium*, vascular wilt, *Vigna*
30 *unguiculata*, race-specific resistance.

31

32 *Fusarium oxysporum* f. sp. *tracheiphilium* (E. F. Sm) Snyder and Hansen is the
33 causal agent of wilt disease in cowpea, resulting in poor stand establishment, poor
34 plant growth (Smith et al., 1999), and resulting yield loss up to 65% (Singh, 2014).
35 The destructive effect of Fusarium wilt on cowpea production justifies research to
36 understand and ameliorate its impact, and for breeding for resistance to the disease.
37 The identification of an effective source of Fusarium wilt resistance, the development
38 of segregating breeding populations and selection of superior breeding lines carrying
39 resistance alleles, coupled with genomic analysis are crucial steps for breeding
40 Fusarium wilt resistant cultivars. Phenotype-based screening for resistance donors
41 and selection of superior breeding lines requires a sensitive screening method with
42 reliable metrics to accurately assess the severity of disease. Reliable disease-
43 phenotyping metrics allow for accurate identification of true phenotypic disease
44 responses (resistant, moderately resistant and susceptible) in screening assays
45 (Luckew et al., 2012). Fusarium wilt resistant and susceptible cowpea genotypes can
46 be easily identified in a germplasm collection or in a segregating population when the
47 trait is under control by one or two genetic factors. However, under quantitative
48 inheritance effective selection for resistant genotypes can be compromised by the
49 lack of an objective screening method; therefore, in these cases quantitative metrics

50 can provide accurate and precise quantification of the severity of disease (Ndeve,
51 2017). In resistant and moderately resistant genotypes, disease severity expressed
52 as the extent of plant wilting can be weakly associated with length of vascular
53 discoloration which is an indicator of the extent of vascular colonization and the
54 damage caused by the fungus (Talboys, 1972; Luckew et al., 2012; Ndeve, 2017).
55 Less objective disease assessment is error prone and can lead to inaccurate
56 association between plant wilting and the extent of vascular discoloration and
57 damage, which could compromise the individual usefulness of each metric in
58 *Fusarium* wilt disease severity assessment and the translation of the results into
59 practical use.

60 Variation in wilt disease phenotypes has been reported in several studies. In
61 common bean and cotton, extensive and severe colonization of the vascular system
62 occurs on susceptible cultivars and breeding lines, and plant wilting is strongly
63 correlated with vascular discoloration (Pastor Corrales and Abawi, 1987; Buruchara
64 and Camacho, 2000; Ulloa et al., 2006). In both crops, some resistant common bean
65 cultivars and cotton breeding lines exhibited mild wilting and discoloration of vascular
66 tissues. In a study by Gao et al. (1994) on eight tomato cultivars screened for
67 resistance to five forms and races of *Fusarium oxysporum* f. sp. *lycopersici*, vascular
68 discoloration was strongly correlated with plant wilting, and there was no evidence of
69 a tolerant reaction. According to Talboys (1972), *Fusarium* wilt tolerant cultivars can
70 be misclassified as resistant cultivars if disease severity is quantified solely based on
71 the external phenotypic wilt reaction because systemic vascular colonization in
72 tolerant cultivars does not necessarily lead to plant wilting. As in cowpea, susceptible
73 common bean and cotton cultivars can display wilt symptoms 5 to 9 days after
74 inoculation, and severe or complete plant wilting and defoliation, which indicates the

75 severity of damage to the vascular system, can occur 14 days after inoculation
76 (Pastor Corrales and Abawi, 1987; Ulloa et al., 2006). Luckew et al. (2012) reported
77 that in lines of soybean from resistant x resistant crosses, the severity of wilt caused
78 by *Fusarium virguliforme* was not correlated with the severity of damage in the root
79 system. These reports indicate that reliance on plant wilting to quantify severity of
80 Fusarium wilt and the use of qualitative and semi-quantitative metrics can
81 compromise the accuracy of wilt disease severity estimation, especially if plant wilt
82 phenotypes are not highly associated with vascular discoloration symptoms as the
83 two traits are under control by distinct mechanisms (Talboys,1972; Luckew et al.,
84 2012)

85 Plant disease severity assessment in many crop species relies mostly on visual
86 assessment based on rating scales (Bock and Nutter, 2011; Chiang et al., 2014). In
87 general, severity of fusariosis is quantified utilizing rating scales in which the external
88 plant phenotypic response (wilting) is used to express the severity of disease.
89 Disease assessment rating scales include ordinal scales of 1 – 5 (Rigert and Foster,
90 1987) 1 – 9 (Van Schoonhoven and Pastor Corrales, 1987; Pastor Corrales and
91 Abawi, 1987), nominal or descriptive scales (resistant and susceptible) (Lv et al.,
92 2014) and nearest percent estimates or ratio scale (Walker, 1930; Palti and Joffe,
93 1971; Joffe et al., 1974; James, 1974; Netzer et al., 1977; Netzer and Weintall, 1980;
94 Sharma et al., 2004; Lv et al., 2014; Chiang et al., 2014). Similarly to nominal scales,
95 ordinal scales are also descriptive; however, the severity of disease is graded using
96 arbitrary classes which indicate distinct levels of severity of disease symptoms (Bock
97 et al., 2010).

98 Other Fusarium wilt disease severity assessment methods have employed
99 estimates of both plant wilt and vascular discoloration phenotypes by using ordinal

100 rating scales of 0 - 5 (Ulloa et al. 2006; Pottorff et al., 2012; Pottorff et al., 2014) or 1
101 – 9 (adapted from Pastor Corrales and Abawi, 1987) (Fall et al., 2001). However,
102 Pastor Corrales and Abawi (1987) and Buruchara and Camacho (2000) used an
103 ordinal rating scale of 1 – 9 to phenotype plant wilting and a nominal or descriptive
104 scale to estimate extent of vascular discoloration. The severity of vascular damage
105 was described as none, light, intermediate and severe. In cotton, Wang et al., 2017
106 assessed the severity of Fusarium wilt disease using an ordinal scale of 0 – 5 to
107 express the severity of plant wilt and a ratio scale (0 – 100%) to express the extent
108 of vascular discoloration.

109 Each of the visual rating scales described above is prone to errors which can
110 compromise the accuracy, precision, repeatability and reproducibility of estimates of
111 Fusarium wilt severity. Errors can limit correct differentiation among resistant,
112 moderately resistant and tolerant plant phenotypes. Indeed, when less experienced
113 individuals use visual rating scales to estimate disease severity, the magnitude of
114 inaccuracy and imprecision in disease severity estimates can be substantial due to
115 evaluator subjectivity, compromising the usefulness of the results for critical research
116 or breeding decisions (Nutter et al., 1993; Bock et al., 2008).

117 This study introduces a novel, objective metric based on the number of necrotic
118 vessels for phenotyping the extent of vascular symptoms incited by Fusarium wilt in
119 cowpea. The relative accuracy of the new metric in expressing severity of wilt is
120 contrasted with vascular discoloration length (%VDL), also an objective metric used
121 to quantify the extent of vascular colonization by Fusarium wilt. The relationships
122 between plant wilting symptoms and the objective metrics are explored. The concept
123 accuracy is defined as the degree of closeness of an estimate to the true or actual
124 value (Nutter et al., 1991), in this study for analytical purpose the true or actual

125 phenotypic value is the plant wilt phenotype, and it is potentially predicted based on
126 the severity of vascular discoloration or the number of necrotic vessels. Therefore,
127 these two parameters are contrasted to determine to what extent their measured
128 values closely reflect the severity of plant wilt (the true value).

129 **MATERIALS AND METHODS**

130 **Plant material.** One $F_{2:3}$ and seven F_2 cowpea populations were used to
131 determine the variability in plant wilting (expressed as a wilt score), vascular
132 discoloration length (expressed as the percent of stem height, %VDL), and vascular
133 necrosis (expressed as number of Fusarium necrotic vessels - NFNV) phenotypes.

134 In brief, to generate the F_2 and $F_{2:3}$ populations, a Fusarium wilt race 4 (Fot4)
135 resistant genotype (Parent A, Table 1) was crossed with three other resistant
136 genotypes (Parents C, E and H; Table 1) to derive F_1 generations of resistant ×
137 resistant populations (populations 5, 6 and 7), and with four susceptible genotypes
138 (Parents B, D, F and G; Table 1) to derive F_1 generations of susceptible × resistant
139 populations (populations 1, 2, 3 and 4). In all crosses, parent A was used as the
140 pollen donor male and all other parents as female. A single F_1 seed of each cross
141 was planted to generate seven F_2 populations (Table 1); in addition, 175 seeds of F_2
142 population 1 (Table 1) were planted to generate an $F_{2:3}$ population (population 8,
143 Table 1). The populations, their sizes and number of plants evaluated per parent
144 (control) are presented (Table 1). The number of plants evaluated per $F_{2:3}$ family
145 varied from 8 to 31 (the average number of plants evaluated per family was 25).

146 The F_2 populations and an $F_{2:3}$ population were used for this study because of
147 their high levels of segregation compared to late generation recombinant inbreeding
148 lines. The levels of segregation in these populations were used to capture a full
149 range of expected phenotypic responses (resistant, intermediate and susceptible) in

150 cowpea screening assays; for example, in cowpea germplasm screenings aimed at
151 identifying novel sources of resistance to Fot4, and in segregating populations
152 carrying potential Fot4 resistance allele combinations. In cowpea breeding programs,
153 phenotyping for Fot4 resistance in earlier generations (e.g. F_{2:3}) is crucial for early
154 plant selection and to capitalize on genotyping resources for effective and efficient
155 plant selection.

156 **Inoculum preparation.** A dried culture of Fot4 isolate T97-30 (isolated from
157 infected cowpea plants in Bakersfield, California), derived from a single spore line
158 was stored at -80 °C on potato dextrose agar (PDA) plates (Pottorff et al., 2014). The
159 dried culture was prepared by culturing the fungus in a shallow and thin layer of PDA
160 in a petri dish. A single 0.5-cm² plug was cut from the dried culture and transferred
161 onto a new petri dish containing fresh PDA amended with 3 mM streptomycin/liter.
162 The culture was incubated at room temperature for 3-4 days under a 16 photoperiod.
163 A fresh 1-cm² plug was aseptically cut from the new culture and placed in a 500 ml
164 Erlenmeyer flask containing freshly prepared and cooled potato-dextrose broth. The
165 flask was incubated in a shaker for 4 days at 30 rpm, and 27 °C under constant light.
166 The broth was filtered through 8 layers of cheesecloth, and the flow-through solution
167 containing spores was collected in a beaker. The spores were counted using a
168 hemocytometer under a light microscope, and the concentration was adjusted to 10⁶
169 microconidia/ml using sterile distilled water. The inoculum was used immediately for
170 plant inoculation.

171 **Growth conditions and infection assays.** The experiments were conducted in a
172 controlled environment greenhouse at the University of California, Riverside (29 to
173 32 °C) under a 14 hours photoperiod. The F₂, F_{2:3} and control genotypes were
174 planted in seedling-trays containing Sungro[®] Horticulture Sunshine mix #2 growing

175 medium (Sun Gro Horticulture Canada Ltd). Following a modified protocol of Rigert
176 and Foster (1987), seven days after planting, the seedlings were uprooted, and the
177 root system washed, clipped to 3 cm length, dipped for 4 minutes into a fresh spore
178 suspension of Fusarium wilt race four (Fot4) containing 10^6 spores/ml water
179 prepared as described above, and transplanted into 0.95 L foam cups (Fig. 1B and
180 1C) containing UC-Mix 3 soil (University of California Riverside,
181 <http://agops.ucr.edu/soil>). The seedlings were watered once per day, and two weeks
182 after inoculation they were fertilized using Osmocote Classic 14-14-14 fertilizer
183 (Everris NA Inc., Dublin, OH).

184 **Evaluation of wilting.** Thirty-five days after inoculation, individual plant stems
185 were cut at the soil line (Fig. 1A), and the plants evaluated for wilting, %VDL, NFNV,
186 plant height (PH) and shoot-weight (SW). Wilting severity was assessed following a
187 0 – 5 categorical rating scale (wilt score) modified from Rigert and Foster (1987) ,
188 where 0 = a healthy plant with no yellowing/wilting symptoms (Fig. 1B – pot 1B); 1 =
189 >0 - 10% of the plant canopy showing yellowing/wilting symptoms (canopy loss up to
190 10%); 2 = >10 - 25% of the canopy showing yellowing/wilting/stunting (canopy loss
191 more than 10 to 25%); 3 = >25 - 50% yellowing/wilting and canopy loss of more than
192 25 to 50%, with a dark-brown spot visible on the base of the petiole and on the
193 petiole scar on the stem; 4 = >50 - 75% yellowing/wilting, severe canopy loss (more
194 than 50 to 75%), with the stem colored green-brownish; and 5 = >75 - 100%, the
195 canopy almost completely to completely lost (more than 75 to 100%), the plant is
196 moribund or dead, with limited green-brownish stem to all brown stem tissue,
197 respectively (Fig. 1C).

198 **Vascular discoloration length (%VDL).** The stem of each plant assessed for
199 wilting was slit open longitudinally with a razor blade from the base to the apex (Fig.

200 1E), and the extent of vascular discoloration from the base was measured using a
201 ruler. The severity of vascular discoloration length (%VDL) was calculated as the
202 ratio between the length of vascular discoloration and the total plant stem height,
203 $(VDL (\%) = \frac{VDL}{PH} \times 100)$.

204 **Vascular necrosis.** After plants were evaluated for wilting and vascular
205 discoloration length, the number of dark-brown discolored vessels (Figs. 1A and 1E)
206 were enumerated in the longitudinally cut stem sections (Fig.1E) to assess the
207 severity of vascular necrosis caused by Fusarium wilt. Enumeration of necrotic
208 vessels on longitudinal cut stem sections is preferable, because on transverse cut
209 stem sections (Figs. 1A) it is hard to visualize clustered-adjacent necrotic vessels. To
210 enumerate necrotic vessels, entire plant stems were cut open along one side, and
211 the stem opened (like opening a book), flattened, placed interior surface up on a
212 porta-trace light box (Gagne Associates, Inc., Binghamton, N. Y.) and the dark-
213 brown vessels counted. In plants with thicker stems, the parenchyma was lightly
214 scalped using a razor blade to facilitate light penetration for better visualization and
215 enumeration of clustered necrotic vessels.

216 **Data analysis.** All analyses were performed using SAS studio version 3.7 (SAS
217 University Edition, SAS, Cary, NC). The association between the plant wilting (wilt
218 score) and vascular discoloration (%VDL), plant wilting and number of Fusarium
219 necrotic vessels (NFNV); and %VDL and NFNV was explored using correlation
220 analysis. Specifically, Pearson's correlation analysis was performed to explore for
221 the association between %VDL and NFNV since both are continuous variables. The
222 relationships between plant wilting and NFNV and between plant wilting and %VDL
223 were explored using Spearman's rank correlation because these relationships
224 involved a categorical and a continuous variable.

225 The relationships between plant wilting (wilt score) and %VDL, and between plant
226 wilting and NFNV were determined using linear regression analysis. Plant wilting (the
227 dependent variable) was regressed against either %VDL or NFNV since plant wilting
228 is a result of vascular occlusion and damage to the plant vascular system (the
229 independent variables were %VDL and NFNV) caused by Fusarium infection. The
230 accuracy of both plant wilting predictors, %VDL and NFNV, were compared using
231 estimated coefficients of determination (R^2) from both regression analyses. Although
232 no cause-effect relationship is expected between %VDL and NFNV, regression
233 analysis was performed to examine their relationship in expressing the severity of
234 vascular damage caused by Fusarium wilt and to corroborate the results of the
235 correlation analysis between these phenotypes.

236 In addition, plant wilting, %VDL and NFNV phenotypic data were tested for
237 several genetic models of segregation between susceptibility – resistance
238 phenotypes to examine segregation distortions using Chi-square goodness-of-fit
239 analysis (Little and Hills, 1978). The Chi-square values were adjusted following
240 Yates correction for continuity (Little and Hills, 1978). The genetic models tested for
241 segregation included a single gene, two dominant genes, and dominant-recessive
242 genes, respectively. The expected segregation ratios between susceptible and
243 resistant phenotypes would be 3:1, 15:1 (9:7) and 13:3, respectively. The ratio 9:7
244 differs from 15:1 in that it includes the interaction between both genes. These
245 segregation ratios were considered based on reports in the literature describing
246 inheritance of resistance to Fusarium wilt in cowpea and other crops, which is
247 controlled by only a few genes (Netzer et al., 1977; Rigert and Foster, 1987; Salgado
248 et al., 1995; Cross et al., 2000; Pottorff et al., 2014). The distinction between
249 susceptible and resistant phenotypic responses was based on the average plant wilt

250 score, %VDL and NFNV of parents and all populations, and the threshold was set at
251 a plant wilt score of < 3 and ≥ 3 (corresponding values for %VDL and NFNV were
252 used as thresholds for these metrics), which indicate plant wilting of $\leq 25\%$ and plant
253 wilting $> 25\%$, respectively.

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RESULTS

258 **Association and relationship between wilt phenotype variables.** All four
259 susceptible parents (CB46, INIA-73, 24-125B-1 and Bambey-21) developed
260 extensive vascular discoloration, with numerous necrotic vessels, wilted and
261 eventually died following infection with Fot4. In contrast, the resistant parents IT93K-
262 503-1, Ecute and FN-2-9-04 did not exhibit noticeable wilting symptoms (Table 2).
263 However, very few vascular vessels (1 to 3) were detected with necrotic symptoms
264 extending 3 to 26% of the plant height.

265 **Correlation analysis:** There was strong association among all the variables in
266 the different populations. The correlation coefficients for the association between the
267 severity of plant wilting and NFNV for populations 1, 2, 3, 4, 5, 6 and 7 were 0.874,
268 0.892, 0.838, 0.941, 0.799, 0.781 and 0.522 (all with $P < 0.00001$), respectively.
269 Correlation coefficients for the association between the severity of plant wilting and
270 %VDL for populations 1, 2, 3, 4, 5, 6 and 7 were 0.892, 0.894, 0.855, 0.922, 0.779,
271 0.769, and 0.513 (all with $P < 0.00001$), respectively. Correlation coefficients for the
272 association between NFNV and %VDL for populations 1, 2, 3, 4, 5, 6 and 7 were
273 0.898, 0.913, 0.968, 0.965, 0.940, 0.916 and 0.884 (all with $P < 0.00001$),
274 respectively. Similarly, with the $F_{2:3}$ population comprising 175 families, correlation

275 coefficients of the associations between the severity of plant wilting and NFNV, and
276 between the severity of plant wilting and %VDL; and between NFNV and %VDL
277 were 0.875, 0.925 and 0.832 (all $P < 0.00001$), respectively.

278 **Regression analysis:** In all F_2 population types (populations 1, 2, 3, 4, 5, 6 and
279 7), the severity of plant wilting was linearly related to NFNV (Figs. 2A – 2G) and
280 %VDL (Figs. S1A – S1G). For NFNV, the coefficient of determination indicated a
281 moderately strong to strong linear relationship ($R^2 = 0.78 – 0.91$). Similarly, the linear
282 relationship between %VDL and plant wilting was moderately strong to strong ($R^2 =$
283 $0.81 – 0.87$). For all F_2 populations, there was a consistently strong linear
284 relationship between %VDL and NFNV ($R^2 = 0.81 – 0.94$). Similarly, for the $F_{2:3}$
285 population comprising 175 families, the linear relationships were strong between the
286 severity of plant wilting and NFNV, and between the severity of plant wilting and
287 %VDL, and between NFNV and %VDL ($R^2 = 0.71, 0.87$ and 0.69 , respectively) (Figs.
288 S3A, B and C, respectively).

289 **Plant selection.** Thresholds for plant selection between resistant and susceptible
290 plants were determined for NFNV and %VDL using the association of both
291 phenotypes with plant wilt. Plants with NFNV phenotypes ranging from 1 to 18 in the
292 F_2 populations (1 to 26 for the $F_{2:3}$ population) had corresponding associated
293 severities of wilt. The threshold for resistant and susceptible wilt phenotypes was
294 fixed at disease score < 3 and ≥ 3 , respectively. This threshold of wilt symptoms was
295 determined based on the average plant wilting across all populations and parental
296 phenotypes. On the wilt rating scale, a score of 3 indicates plants with > 25 to 50%
297 yellowing/wilting and > 25 to 50% of the canopy wilted or lost. Based on this wilting
298 threshold, critical threshold values were determined for NFNV and %VDL, equivalent
299 to visual estimates of the severity of wilting (Tables 3 and 4).

300 The F₂ plants (populations 1, 2, 3, 4, 5, 6 and 7) barely exhibiting visible wilt
301 symptoms the mean wilt score was 0.1 to 0.5, or less than 10% of the plant wilted.
302 The NFNV score for these plants ranged from 1 to 3, and the mean %VDL from 13.2
303 to 25.2%. Plants with a mean wilt score of 1.6 - 2.1 (approximately > 10 to 25% of
304 the plant wilted) had an NFNV of 4 to 5, which corresponded to a mean %VDL of
305 29.6 – 32.7% (Table 3). Wilting of 50% (wilt score of 2.8 - 2.9) was observed in
306 plants with an NFNV of 6 or 7, and a corresponding mean %VDL of 45.2 to 49.0%;
307 whereas plants ≥ 75% wilted (a wilt score of 3.4 to 4.6) had an NFNV ≥ 8, which
308 corresponded to a %VDL of 46.1 to 76.7%. The total number of vessels in each plant
309 was not enumerated, but it was observed that even the highly susceptible genotypes
310 with 10 to 18 necrotic vessels in the F₂ generation, had some vessels that were not
311 necrotic (Table 3).

312 Also, the F_{2:3} population (population 8), which comprised 175 families (on average
313 25 plants/family) showed evidence of associations among assessment methods.
314 Plants with mean wilt scores of 0.3 to 1.1 (> 0 to 10% wilted) had an NFNV of 1 – 3
315 and a mean %VDL ranging from 5.5 to 16.7% (Table 4). Plants with wilt scores of 1.6
316 to 2.1 (> 10 to 25% wilted) had an NFNV of 4 to 6 and a mean %VDL ranging from
317 27.1 to 36.2, respectively. Plants that were 50% wilted (wilt score of 2.5 to 3.2) had
318 an NFNV of 7 to 9, which corresponded to a mean %VDL of 46.1 to 55.4%,
319 respectively. Plants with wilt score of 3.8 (≥ 75% wilted) had and NFNV ≥ 10, and a
320 %VDL ≥ 69.9.

321 **Segregation distortion.** Two F₂ populations (susceptible × resistant population 1
322 and resistant × resistant population 5) were analyzed to determine the levels of
323 segregation distortion observed for each Fusarium wilt severity assessment metric:
324 wilting, vascular discoloration length (%VDL) and number of Fusarium necrotic

325 vessels (NFNV). A cut-off between resistant and susceptible phenotypes was set at
326 a wilt score of < 3, a %VDL < 45 and an NFNV count < 6. These thresholds were
327 based on 50% plant wilt and the phenotypic responses of the F₂ population to wilting,
328 vascular discoloration length and vascular necrosis (Table 3).

329 The best fitting segregation ratio (Resistant: Susceptible) for wilting (206:158),
330 %VDL (225:139) and NFNV (208:156) in F₂ population 1 was 9:7 (Table 5); however,
331 this was significant only for wilt score ($X^2 = 0.00$, $P = 0.99$) and NFNV ($X^2 = 0.08$, $P =$
332 $0.75 - 0.90$). Segregation distortion was higher for %VDL compared to the wilt score
333 and NFNV, and the differences between observed and expected phenotypic
334 responses were 19.75, 0.75 and 2.75, respectively. In the resistant × resistant F₂
335 population 5, the best fit segregation ratio for wilt score (251:72), %VDL (242:81) and
336 NFNV (251:72) was 13:3. This model was significant for the wilt score and NFNV (X^2
337 $= 2.43$, $P = 0.10 - 0.25$ for both phenotypic metrics), but it was not significant for
338 %VDL ($X^2 = 8.08$, $P < 0.01$). Also, in F₂ population 1, both wilt score and NFNV had
339 lower segregation distortion (10.94) compared to %VDL (19.94).

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DISCUSSION

342 The plant wilt score was highly correlated with both vascular discoloration length
343 (%VDL) and number of necrotic vessels (NFNV) in the seven F₂ populations and one
344 F_{2:3} population of cowpea. Associations between wilting and vascular discoloration
345 induced by *Fusarium* has been reported in tomato, common bean and cotton (Gao et
346 al., 1994; Buruchara and Camacho, 2000; Ulloa et al. 2006); however, in soybean,
347 these phenotypic responses were not associated with one another (Luckew et al.,
348 2012). In this study, wilt response was correlated with both %VDL and NFNV, and
349 most of the variability in plant wilt phenotypes was accounted for by both %VDL and

350 NFNV, as indicated by the coefficients of determination (R^2 all ≥ 0.71); however, the
351 use of only one of the three phenotypes (plant wilt, %VDL or NFNV) alone may not
352 be completely effective in measuring disease severity in the cowpea – *Fusarium*
353 pathosystem and quite possibly in other plant wilt pathosystems. Other factors
354 including germplasm and pathogen isolate origin, the anatomy of the vascular
355 system and measurement errors may undermine the relationship, as indicated by
356 segregation distortion. For example, in studies on *Fusarium* wilt of cotton,
357 phenotyping based on a combination of plant wilting and %VDL has proved effective
358 (Ulloa et al., 2006; Wang et al., 2017). In cowpea, recently we have demonstrated
359 through a series of genetic studies and quantitative trait loci mapping that
360 determinants for resistance to wilt caused by *Fusarium oxysporum* f. sp.
361 *tracheiphilum* race 4 in accession FN-2-9-04 (the resistant parent used to develop
362 populations used in this study) are located on two chromosomes, and resistance to
363 %VDL and NFNV are co-located with resistance to wilting on only one of the
364 chromosomes (Ndeve, 2017). This finding indicated that plant wilting may be a
365 complex trait under control by several genes, some of which may be associated with
366 resistance to vascular discoloration and to the proliferation in the number of necrotic
367 vessels, which are associated with the development of the wilting phenotype.

368 The phenotypes %VDL and NFNV were highly correlated; however, %VDL
369 expresses the extent of vertical vascular discoloration in the plant stem, but it does
370 not take into account the number of necrotic vessels in the plant stem required to
371 trigger the plant wilt symptoms resulting from occluded and damaged vessels. For
372 example, in some cases, infected plants have one to four discolored vessels
373 extending >50% of the plant height, but they will have no visual symptoms of wilt;
374 therefore, %VDL will not be correlated with plant wilt phenotype and %VDL

375 measurements will overestimate the severity of vascular damage. In this situation, it
376 is important to determine the number of discolored vessels for accurate phenotype-
377 based plant selection. Vascular discoloration length (%VDL) and NFNV were
378 contrasted to determine how closely their values reflect the severity of plant wilt
379 (True value) and their usefulness for plant selection for resistance to Fusarium wilt
380 disease. The analyses showed that in both the F_2 and $F_{2:3}$ test populations a
381 vascular discoloration length of 45% was associated with a wilt score of 3 (50%), and
382 this wilt score was associated with a count of 6 to 7 necrotic vessels. Both the %VDL
383 and NFNV are quantitative metrics that provide objective measurement (as opposed
384 to a subjective estimate) of Fusarium wilt severity in cowpea plants, and the data can
385 be analyzed using parametric statistics (Campbell and Neher, 1994). Conversely,
386 rating the extent of vascular discoloration and wilting symptoms using a categorical
387 scale (e.g., 0-5) with corresponding disease severity classes indicating percent
388 vascular discoloration or percent of plant wilting (0, 1, 2, 3, 4 and 5 = 0, 10, 25, 50,
389 75 and 100%) (Pottorff et al., 2012; Pottorff et al., 2014) can lead to errors in disease
390 assessment due to subjectivity inherent among raters, which can be exacerbated by
391 inexperienced or less accurate disease evaluators (Nutter et al., 1993; Bock et al.,
392 2010). Visual assessment of the severity of Fusarium wilt symptoms relies on visual
393 rating scales that express the severity of plant wilting, and the externally visible
394 symptoms of wilt are a direct consequence of damage to the vascular system. The
395 modifications to the process of Fusarium wilt disease assessment described in this
396 study allow for quantitative measurement of severity of vascular colonization by a
397 combination of length of vascular discoloration (%VDL) and enumeration of necrotic
398 vessels (NFNV). Using NFNV was less error prone compared to measuring the
399 length of vascular discoloration, as indicated by the segregation distortion between

400 the observed and expected numbers of resistant and susceptible plants. Because
401 symptoms of Fusarium wilt-induced vascular damage are generally similar in a range
402 of crop plant species, including cotton (Ulloa et al., 2006; Wang et al., 2017) and
403 tomato (Buruchara and Camacho, 2000), the protocol described in this study may be
404 usefully applied to assess Fusarium wilt disease severity on these crops and in other
405 plant-Fusarium wilt pathosystems. Enumerating the number of necrotic vessels in
406 infected plants is time consuming, which is an important consideration in breeding
407 programs where phenotyping large numbers of plants is required. But both NFNV
408 and %VDL may be amenable to measurement using digital-image phenotyping
409 technology (Nutter and Schultz, 1995; Bock and Nutter, 2011; Barbedo, 2013; Mutka
410 and Bart, 2015; Mahlein, 2016). Image analysis software can be programmed to
411 count the number and the length of vascular necrotic vessels (NFNV and %VDL) in
412 images of longitudinal plant sections. Although this method has not been explored it
413 could reduce the labor required and may provide added accuracy and repeatability of
414 disease severity estimates in Fusarium wilt phenotyping, which are crucial for
415 effective plant selection and breeding for Fusarium wilt resistance.

416 The accuracy and precision of digital-image based plant disease phenotyping
417 varies with pathosystem (Bock and Nutter, 2011; Mutka and Bart, 2015). For
418 example, measurements of disease severity of sunflower blight and oat leaf rust
419 using digital-image analysis were overestimated compared to visual estimates
420 (Tucker and Chakraborty, 1997). Similar results were reported by Olmstead et al.
421 (2001) when assessing powdery mildew disease severity on sweet cherry, whereas
422 radiometric assessment of dollar spot severity on bentgrass was more precise than
423 visual assessment (Nutter et al., 1993). Bock et al. (2008) reported that digital-image
424 phenotyping of citrus canker on grapefruit leaves was more consistent and accurate

425 when compared to visual disease assessment. Thus, the accuracy, precision,
426 repeatability and reproducibility of image-based plant disease assessments varies
427 among pathosystems and are likely affected by the symptoms, characteristics of the
428 image, and the conditions under which the images were captured (Bock and Nutter,
429 2011).

430 This study was based on F_2 and $F_{2:3}$ populations because the segregation and
431 zygosity at these generations enabled a broad assessment of the variability of the
432 phenotypic variables of interest, which are less apparent in advanced highly inbred
433 lines (e.g. F_8 or F_{10}). In plant breeding programs, plant selections for wilt resistance
434 traits must be strategized to avoid loss of breeding material to Fusarium wilt
435 infection, including through destructive assessments, such as the ones described in
436 this study. In early generations, for example F_2 populations, plants are not inoculated
437 with Fusarium wilt and leaf samples can be collected for DNA extraction and
438 subsequent genotyping using previously characterized molecular markers (Ndeve,
439 2017). Each F_2 plant is managed to produce enough seed for the next generation
440 ($F_{2:3}$) for conducting further Fusarium wilt resistance phenotyping. After phenotyping
441 the $F_{2:3}$ for plant wilting, %VDL and/or NFNV, the phenotypic data are associated
442 with genotypic data through marker-trait association analysis. This approach
443 associates the plant phenotype (plant wilting, %VDL and/or NFNV) with the specific
444 target trait alleles to allow the identification and selection of plants of interest (these
445 materials trace back to their $F_{2:3}$ seed stocks). Also, this approach enables location of
446 the resistance gene(s) associated with the phenotypes in the plant genome and
447 reduces the time required for the breeding cycle. Since all phenotyped $F_{2:3}$ plants are
448 either killed by Fusarium wilt disease or destroyed during plant phenotyping, the data
449 from marker-trait association analysis enables tracing the identity and selection of

450 plants of interest (resistant) back to their original seed stocks, which can be used for
451 subsequent advancement of selected breeding material. Similar approaches using
452 phenotyping and genotyping on different generations (Zhang and Xu, 2004) can be
453 employed including marker-assisted backcrossing and marker-assisted recurrent
454 selection. In this study, the variation in phenotypic response (wilt score, NFNV and
455 %VDL) was a desirable condition in that it allowed detection of extreme phenotypes
456 (parental), and intermediate phenotypes resulting from quantitative inheritance and
457 heterozygosity. In segregating breeding populations this phenotypic variation
458 provides a basis for understanding the genetic control of the traits including the
459 number, dominance and additive effects of the genes involved.

460 This study introduces the number of Fusarium necrotic vessels (NFNV) as a
461 novel quantitative metric to measure the severity of vascular damage caused by
462 Fusarium wilt disease of cowpea; the strong correlation of NFNV with vascular
463 discoloration length (%VDL), a metric used to measure the severity of vascular
464 damage, showed that both metrics provide accurate measurements of the severity of
465 plant vascular damage under Fusarium wilt disease infection. Also, the strong
466 correlation between NFVN and %VDL indicated that either of these metrics can be
467 used to phenotype plant vascular damage; however, when using %VDL to
468 phenotype vascular damage, plants showing one to four discolored vessels that
469 extend > 50% of the plant height may cause segregation distortion of the ratio
470 between the observed and expected number of plants resistant and susceptible to
471 vascular damage, respectively. In addition, the linear relationship between both
472 vascular metrics and plant wilting phenotype, also provided evidence of the accuracy
473 of NFVN and %VDL as a gauge of the severity of vascular damage and to predict
474 plant wilt phenotype resulting from Fusarium wilt infection. When searching for novel

475 sources of resistance to Fusarium wilt in germplasm collections, it is appropriate to
476 phenotype plants by both plant wilting and vascular damage (NFVN or %VDL)
477 because high phenotypic variation is often present in these materials, allowing
478 identification of resistant, susceptible and partially resistant genotypes. These
479 variations in Fusarium wilt disease phenotypic responses may indicate to some
480 extent a weak correlation between plant wilting and vascular phenotypes. Also,
481 unless the relationship between plant wilting and vascular phenotypes is known in
482 the parental genotypes, plants should be phenotyped for both plant wilt phenotype
483 and vascular phenotype (NFNV or %VDL). Based on this study, both NFNV and
484 %VDL can be used in breeding programs in early or late generation plant
485 phenotyping, providing objective quantification of vascular wilting.

486

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LITERATURE CITED

494 Campbell, C. L., and Neher, D. A. 1994. Estimating Disease Severity and Incidence.
495 In: Epidemiology and Management of Root Diseases. Springer, Berlin,
496 Heidelberg. https://doi.org/10.1007/978-3-642-85063-9_5.
497 Cross, H., Brick, M. A., Schwartz, H. F., Panella, L. W., Byrne, P. F. 2000.
498 Inheritance of resistance to fusarium wilt in two common bean races. Crop
499 Science. 40: 954–958.
500 Barbedo, J. G. A. 2013. Digital image processing techniques for detecting,
501 quantifying and classifying plant diseases. SpringerPlus 2:660.

- 502 Bock, C. H., Parker, P. E., Cook, A. Z., and Gottwald, T. R. 2008. Visual rating and
503 the use of image analysis for assessing different symptoms of citrus canker on
504 grapefruit leaves. *Plant Disease* 92:530-541.
- 505 Bock, C. H., Poole, G. H., Parker, P. E., and Gottwald, T. R. 2010. Plant disease
506 severity estimated visually, by digital photography and image analysis, and by
507 hyperspectral imaging. *Critical Reviews in Plant Sciences* 29: 2, 59-107.
- 508 Bock, C.H., and Nutter, F. W. Jr. 2011. Detection and measurement of plant disease
509 symptoms using visible-wavelength photography and image analysis. *Plant*
510 *Pathology and Microbiology Publications*. 67.
511 https://lib.dr.iastate.edu/plantpath_pubs/67.
- 512 Buruchara, R. A., and Camacho, L. 2000. Common bean reaction to *Fusarium*
513 *oxysporum* f. sp. *phaseoli*, the cause of severe vascular wilt in central Africa. *J.*
514 *Phytopathology* 148:39-45.
- 515 Fall, A., Byrne, P., Jung, G., Coyne, D., Brick, M., and Schwartz, H. 2001. Detection
516 and mapping of a major locus for *Fusarium* wilt resistance in common bean.
517 *Crop Science* 41(5):1494–1498.
- 518 Gao, H., Beckman, C., H., and Mueller, W. C. 1995. The rate of vascular colonization
519 as a measure of the genotypic interaction between various cultivars of tomato
520 and various formae or races of *Fusarium oxysporum*. *Physiological and*
521 *Molecular Plant Pathology*. 46:29-43.
- 522 James, W. C. 1974. Assessment of plant diseases and losses. *Annual Review of*
523 *Phytopathology* 12:27-48.
- 524 Joffe, A. Z., Palti, J., and Arbel-Sherman, R. 1974. *Fusarium oxysporum* Schlecht in
525 Israel. *Phytoparasitica* 2:91-107.
- 526 Chiang, K.-S., Liu S.-C., Bock, C. H., and Gottwald, T. R. 2014. What interval
527 characteristics make a good categorical disease assessment scale?
528 *Phytopathology* 104:575-585.
- 529 Little, T. M., and Hills, F. J. 1978. *Agricultural experimentation – design and analysis.*
530 *California (CA): Wiley.*
- 531 Luckew, A. S., Cianzio, S. R., and Leandro, L. F. 2012. Screening method for
532 distinguishing soybean resistance to *Fusarium virguliforme* in resistant x
533 resistant crosses. *Crop Science*. 52:2215–2223.
- 534 Lv, H., Fang, Z., Yang, L., Lin, Y., Wang, Q., Liu, Y., Zhuang, M., Zhang, Y., Yang,
535 Y., Xie, B., Liu, B., Liu, J., Kan, J., and Wang, X. 2014. Mapping and analysis of
536 a novel candidate *Fusarium* wilt resistance gene FOC1 in *Brassica oleracea*.
537 *BioMed Central Genomic* 15:1094.
- 538 Mutka, A., and Bart, R. 2015. Image-based phenotyping of plant disease symptoms.
539 *Frontiers in Plant Sciences* 5, doi: 10.3389/fpls.2014.00734.
- 540 Mahlein, A.-K. 2016. Plantdisease detection by image sensors – parallels and
541 specific demands for precision agriculture and plant phenotyping. *Plant Disease*
542 100(2):241-251.
- 543 Netzer, D., Niego, S., and Galun, E. 1977. A dominant gene conferring resistance to
544 *Fusarium* wilt in cucumber. *Phytopathology* 67: 525-527.
- 545 Netzer, D., and Weintall, C. H. 1980. Inheritance of resistance in watermelon to race
546 I of *Fusarium oxysporum* f. sp. *niveum*. *Plant Disease* 64:853-854.
- 547 Ndeve, A. D. 2017. Genetics of resistance to root-knot nematode and *Fusarium* wilt
548 In cowpea germplasm from Mozambique, Ph. D. dissertation, Dept. Microbiology and
549 Plant Pathology, University of California, Riverside, 2017.
- 550 Nutter, F. W., Jr., Teng, P. S. and Shokes, F. M. 1991. Disease assessment terms
551 and concepts. *Plant Disease* 75(11):1187-1188.

- 552 Nutter, F. W. Jr, Gleason, M. L., Jenco, J. H., and Christians, N. C. 1993. Assessing
553 the accuracy, intra-rater repeatability, and interrater reliability of disease
554 assessment systems. *Phytopathology* 83:806–12.
- 555 Nutter, F. W., Jr. and Shultz, P. M. 1995. Improving the accuracy and precision of
556 disease assessments: selection of methods and use of computer-aided training
557 programs, *Canadian Journal of Plant Pathology* 17:(2)174-184.
- 558 Olmstead, J. W., Lang, G. A., and Grove, G. G. 2001. Assessment of severity of
559 powdery mildew infection of sweet cherry leaves by digital image analysis.
560 *Horticultural Science* 36:107–111.
- 561 Pastor Corrales, M. A., and Abawi, G. S. 1987. Reaction of selected bean
562 germplasm to infection by *Fusarium oxysporum* f. sp. *phaseoli*. *Plant Disease*
563 71:990-993.
- 564 Palti, J., and Joffe, A. Z. 1971. Causes of the Fusarium wilts of cucurbits in Israel
565 and conditions favoring their development. *Phytopathologische Zeitschrift*. 70:31-
566 42.
- 567 Pottorff, M. O., Wanamaker, S., Ma, Y. Q., Ehlers, J. D., Roberts, P. A., and Close,
568 T. J. 2012. Genetic and physical mapping of candidate genes for resistance to
569 *Fusarium oxysporum* f. sp. *tracheiphilum* race 3 in cowpea [*Vigna unguiculata*
570 (L.) Walp]. *PLoS ONE* 7(7):e41600. doi:10.1371/journal.pone.0041600.
- 571 Pottorff, M. O., Li, G., Ehlers, J. D., Close, T. J., and Roberts, P. A. 2014. Genetic
572 mapping, synteny, and physical location of two loci for *Fusarium oxysporum* f.
573 sp. *tracheiphilum* race 4 resistance in cowpea [*Vigna unguiculata* (L.) Walp].
574 *Molecular Breeding* 32(4):doi 10.1007/s11032-013-9991-0.
- 575 Rigert, K. S., and Foster, K. W. 1987. Inheritance of resistance to two races of
576 Fusarium wilt in three cowpea cultivars. *Crop Science* 27: 220-224.
- 577 Salgado, M. O., Schwartz, H. F., Brick, M. A. 1995. Inheritance of resistance to a
578 Colorado race of *Fusarium oxysporum* f.sp. *phaseoli* in common beans. *Plant*
579 *Disease* 79: 279-281.
- 580 Sharma, K. D., Winter, P., Kahl, G., and Muehlbauer, F. J. 2004. Molecular mapping
581 of *Fusarium oxysporum* f. sp. *ciceris* race 3 resistance gene in chickpea.
582 *Theoretical Applied Genetics* 108:1243–1248.
- 583 Singh, B. B., 2014. Cowpea: the food legume of the 21st century. Madison, WI: Crop
584 Science Society of America, Inc. doi: [10.2135/2014.cowpea](https://doi.org/10.2135/2014.cowpea)
- 585 Smith, S. N., Helms, D. M., and Temple, S. R. 1999. The distribution of Fusarium wilt
586 of black-eyed cowpeas within California caused by *Fusarium oxysporum* f. sp.
587 *tracheiphilum* race 4. *Plant Disease* 83 (7): 694.
588 <http://dx.doi.org/10.1094/PDIS.1999.83.7.694C>.
- 589 Talboys, P. W. 1972. Resistance to vascular wilt fungi. *Proceeding of the Royal*
590 *Society of London. Series B.* 181:319-332.
- 591 Tucker, C.C., and Chakraborty, S. 1997. Quantitative assessment of lesion
592 characteristics and disease severity using digital image processing. *Journal of*
593 *Phytopathology* 145: 273–278.
- 594 Ulloa, M., Hutmacher, R. B., Davis, R. M., Wright, S. D., Percy, R., and Marsh, B.
595 2006. Breeding for Fusarium wilt race 4 resistance in cotton under field and
596 greenhouse conditions. *Journal of Cotton Science* 10(2):129-136.
- 597 Van Schoonhoven, A., and Pastor Corrales, M. A. 1987. Standard system for the
598 evaluation of bean germplasm. Centro Internacional de Agricultura Tropical, Cali,
599 Colombia.
- 600 Walker, J. C. 1930. Inheritance of Fusarium resistance in cabbage. *Journal of*
601 *Agricultural Research*. 40:721–45.

- 602 Wang, C., Ulloa, M., Duong, T., and Roberts, P. A. 2017. QTL mapping of multiple
603 independent loci for resistance to *Fusarium oxysporum* f. sp. *vasinfectum* races
604 1 and 4 in an interspecific cotton population. *Phytopathology*
605 <https://doi.org/10.1094/PHYTO-06-17-0208-R>.
606 Zhang, Y. M., and Xu, S. Z. 2004. Mapping quantitative trait loci in F₂ incorporating
607 phenotypes of F₃ progeny. *Genetics* 166:1981–1993.
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TABLES

TABLE 1. Cowpea population designations, crosses, population size, and number of plants per parent (control) evaluated in the *Fusarium* wilt assays.

N ^o	Cross	Gen	Size	Parents (controls)							
				Number of Plants							
				A	B	C	D	E	F	G	H
1	CB46/FN-2-9-04	F ₂	364		39						
2	INIA-73/FN-2-9-04	F ₂	353				28				
3	24-125B-1/FN-2-9-04	F ₂	209						35		
4	Bambey-21/FN-2-9-04	F ₂	120	20						16	
5	CB27/FN-2-9-04	F ₂	323			18					
6	IT93K-503-1/FN-2-9-04	F ₂	363					21			
7	Ecute/FN-2-9-04	F ₂	145								25
8	CB46/FN-2-9-04	F _{2:3}	175	10	22		27		5	6	12

613 N^o = population number; Gen = generation; parents A, B, C, D, E, F, G, H = FN-2-9-04,
614 CB46, CB27, INIA-73, IT93K-503-1, 24-125B-1, Bambey-21 and Ecute, respectively.
615

616

617 TABLE 2. Symptom development in parental genotypes (controls) of cowpea
618 including wilting and development of vascular necrosis induced by *Fusarium*
619 *oxysporum* f. sp. *tracheiphilum* race 4 (Fot4).
620

Response	Parents	Plant wilting score (0-5)	Vascular discoloration length (%)	Number of necrotic vessels in stem longitudinal - section
Susceptible	CB46	4.8 ± 0.0	95.9 ± 0.0	13.2 ± 0.0
	INIA-73	4.2 ± 0.3	68.8 ± 6.5	7.1 ± 0.4
	24-125B-1	4.6 ± 0.1	84.5 ± 5.2	14.3 ± 1.0
	Bambey-21	5.0 ± 0.0	100.0 ± 0.0	18.0 ± 0.0
	Mean ± ^a SE	4.7 ± 0.1	87.3 ± 2.9	13.1 ± 0.4
Resistant	CB27	0.3 ± 0.3	7.1 ± 3.5	0.7 ± 0.5
	IT93K-503-1	0.4 ± 0.1	16.2 ± 2.3	1.7 ± 0.4
	Ecute	0.8 ± 0.0	25.7 ± 2.2	2.5 ± 0.2
	FN-2-9-04	0.0 ± 0.0	2.9 ± 1.5	0.3 ± 0.2
	Mean ± ^a SE	0.4 ± 0.1	12.9 ± 2.4	1.3 ± 0.3

621 ^a ± SE = standard error

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627 TABLE 3. The number of Fusarium necrotic vessels (NFNV), the plant wilt symptom
 628 score and the extent of vascular discoloration (%VDL) observed among seven F₂
 629 populations of cowpea inoculated with *Fusarium oxysporum* f. sp. *tracheiphilum* race
 630 4 (Fot4).
 631

		NFNV									
		1	2	3	4	5	6	7	8	9	10 - 18
		^a Number of plants									
Variable	^b Pop.	31-68	7-42	4-26	2-23	3-11	1-8	2-12	1-10	2-6	42-128
Plant wilting (score, 0-5)	1	0.2	0.2	0.6	1.9	0.9	2.3	2.9	2.9	2.8	4.5
	2	0.1	0.5	0.7	1.8	1.6	2.6	2.8	3.0	^c -	4.7
	3	0.2	0.2	0.4	1.7	2.5	2.2	2.0	4.0	1.0	4.5
	4	0.0	0.3	0.6	2.0	2.3	4.0	2.8	4.0	3.5	4.0
	5	0.1	0.3	0.6	1.1	2.5	3.3	3.5	2.6	4.5	4.9
	6	0.1	0.2	0.5	2.0	2.5	3.3	3.1	3.3	3.5	4.7
	7	0.0	0.0	0.0	0.5	2.3	2.0	^c -	4.0	4.7	^c -
	Mean		0.1	0.3	0.5	1.6	2.1	2.8	2.9	3.4	3.3
Standard Error		0.0	0.1	0.1	0.2	0.2	0.3	0.4	0.2	0.5	0.1
%VDL	1	16.1	18.7	27.8	34.4	32.3	44.6	51.1	43.8	44.3	88.5
	2	18.9	25.9	28.3	34.7	41.5	45.0	45.6	38.8	^c -	91.5
	3	11.4	14.0	24.8	24.6	29.2	31.2	46.0	42.5	32.4	58.4
	4	7.3	16.6	21.6	28.2	33.9	69.5	46.8	58.9	42.7	34.8
	5	19.5	26.5	30.0	38.8	2.5	56.9	49.9	61.2	75.4	97.6
	6	14.5	19.4	29.2	34.5	41.3	48.2	54.3	45.6	43.7	89.3
	7	4.7	8.9	14.7	11.8	47.9	20.8	^c -	32.0	79.0	^c -
	Mean		13.2	18.6	25.2	29.6	32.7	45.2	49.0	46.1	52.9
Standard Error		2.1	2.4	2.1	3.5	5.6	6.0	7.1	4.0	7.3	13.9

632 ^aThe number of plants identified within each NFNV varied among seven populations, and
 633 each NFNV was associated with a wilt score and extent of vascular discoloration in each
 634 population. The NFNV among populations varied from 1 to 18.

635 ^bPop = populations, indicate in the second column; Pop 1 = CB46/FN-2-9-04, 2 = INIA-
 636 73/FN-2-9-04, 3 = 24-125B-1/FN-2-9-04, 4 = Bambey-21/FN-2-9-04, 5 = CB27/FN-2-9-04, 6
 637 = IT93K-503-1/FN-2-9-04 and 7 = Ecute/FN-2-9-04.

638 ^cindicates no plant was identified within that NFNV class.
 639

640 TABLE 4. The number of Fusarium necrotic vessels (NFNV), the plant wilt symptom
 641 score and the extent of vascular discoloration (%VDL) observed in the F_{2:3}
 642 population (Population 8, Table 1) of cowpea inoculated with *Fusarium oxysporum f.*
 643 *sp. tracheiphilum* race 4 (Fot4).
 644

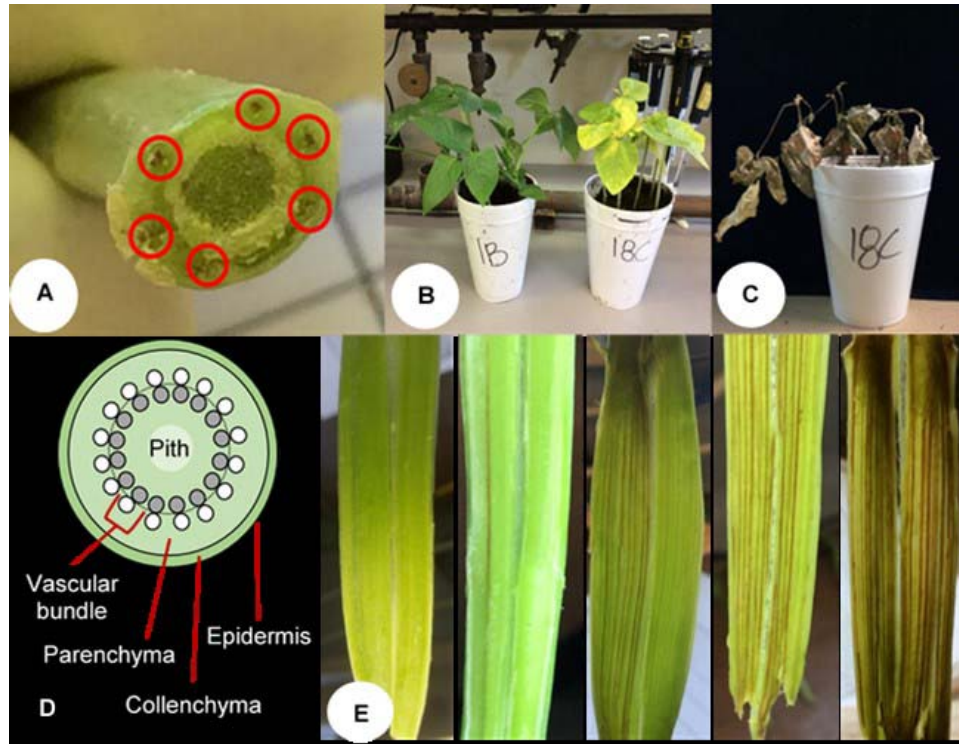
		NFNV									
		1	2	3	4	5	6	7	8	9	10 - 26
		^a Number of families									
Variable		13	8	11	14	20	16	13	16	19	45
Plant wilting (score, 0-5)	Mean	0.3	0.8	1.1	1.8	1.6	2.1	3.2	2.5	3.0	3.8
	Standard Error	0.1	0.2	0.1	0.2	0.1	0.1	0.6	0.2	0.1	0.1
%VDL	Mean	5.5	12.8	16.6	27.1	28.0	36.2	55.4	46.1	50.8	69.9
	Standard Error	0.8	2.5	3.2	3.6	1.7	2.8	15.7	2.7	2.3	2.2

645 ^aThe number of families identified among the NFNV varied from 8 to 45. The NFNV in the
 646 F_{2:3} populations varied from 1 to 26.

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 649 TABLE 5. Segregation for wilting (wilt score), vascular discoloration length (%VDL)
 650 and number of Fusarium necrotic vessels (NFNV) in two F₂ populations (population 1
 651 = susceptible × resistant; population 5 = resistant × resistant) of cowpea inoculated
 652 with *Fusarium oxysporum f. sp. tracheiphilum* race 4 (Fot4).
 653

Variable	Response	Population 1				Population 5			
		^a O	^b E (9:7)	^c O-E	^d X ²	^a O	^b E (13:3)	^c O-E	^d X ²
Wilt score	Resistant	206	204.75	0.75	0.00	251	262.44	10.94	0.46
	Susceptible	158	159.25	0.75	0.00	72	60.56	10.94	1.98
	Total	364	364	1.5	0.00	323	323	21.88	2.43
%VDL	Resistant	225	204.75	19.75	1.91	242	262.44	19.94	1.51
	Susceptible	139	159.25	19.75	2.45	81	60.56	19.94	6.56
	Total	364	364	39.50	4.35	323	323	39.88	8.08
NFNV	Resistant	208	204.75	2.75	0.04	251	262.44	10.94	0.46
	Susceptible	156	159.25	2.75	0.05	72	60.56	10.94	1.98
	Total	364	364	5.50	0.08	323	323	21.88	2.43

654 ^aO and ^bE = observed and expected number of resistant or susceptible plants (values in
 655 brackets are the significant expected model ratios between resistant and susceptible plants);
 656 ^cO-E = deviation from expected value;
 657 ^dX² = chi-square; E (R:S) = expected model ratio.
 658



659

660 **Fig.1.** Symptoms of Fusarium wilt incited by *Fusarium oxysporum* f. sp.
661 *tracheiphilum* race 4 (Fot4) inoculated on cowpea plants. **A**, resistant and
662 susceptible plants (pot 1B and 18C, respectively) 20 days after inoculation; **B**,
663 susceptible plants 35 days after inoculation; **C**, cross-section of cowpea plant stem
664 showing vascular discoloration highlighted in circles 35 days after inoculation; **D**,
665 schematic of stem anatomy of a typical dicotyledon plant; gray and white circles =
666 xylem and phloem, respectively; **E**, longitudinal stem sections of cowpea F₂ lines
667 segregating for resistance 35 days after inoculation; the stems on the far left and far
668 right are of resistant and susceptible plants showing no (left) and severe (right)
669 vascular discoloration, respectively.

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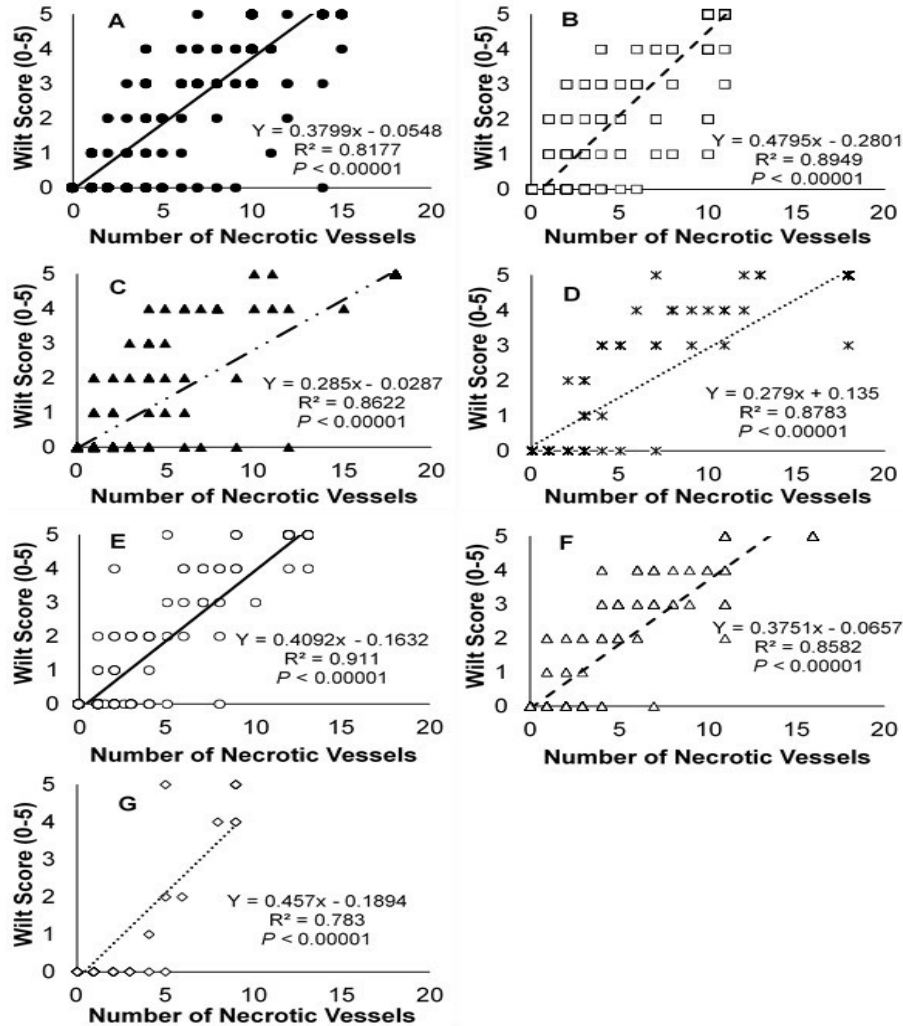
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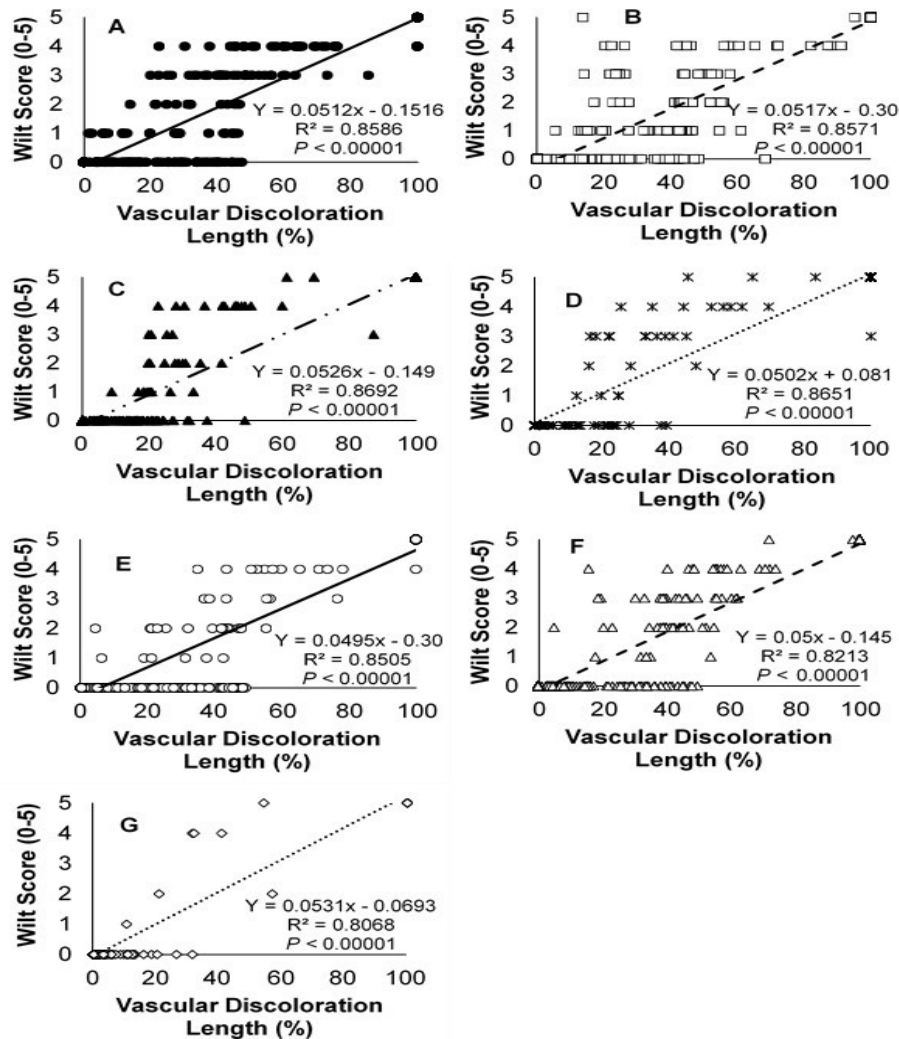


680

681 **Fig. 2.** Relationship between wilt score and number of necrotic vessels in seven F_2
 682 populations (A, B, C, D, E, F and G). **A, B, C** and **D** (populations 1, 2, 3 and 4) –
 683 susceptible x resistant crosses; and **E, F** and **G** (populations 5, 6 and 7) – resistant x
 684 resistant crosses. The linear regression models describe the dependence of plant
 685 wilt on the number of necrotic vessels. The coefficient of determination, R^2
 686 describes the proportion of variance of plant wilting explained by the number of
 687 necrotic vessels. The P -value indicates whether the regression model was
 688 significant.

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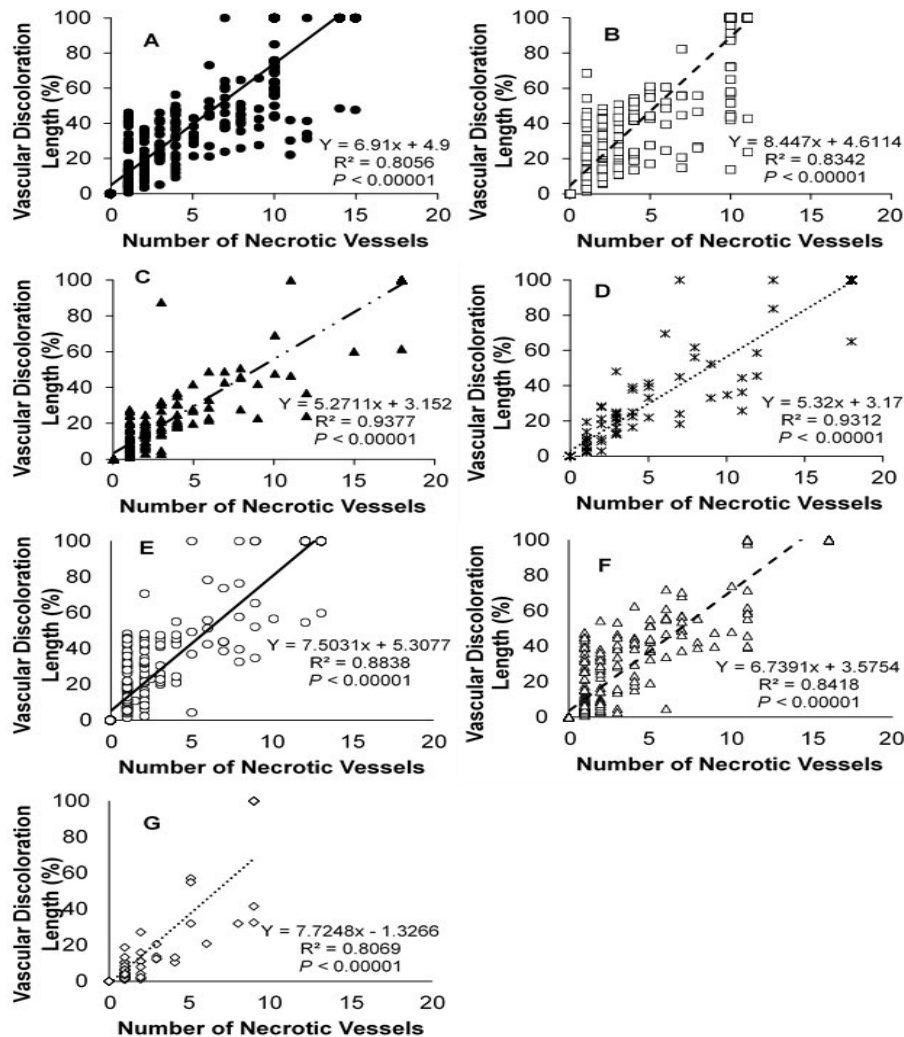
691

692 **S1.** Relationship between wilt score and vascular discoloration length in seven F₂
 693 populations. **A, B, C** and **D** (populations 1, 2, 3 and 4) – susceptible x resistant
 694 crosses; **E, F** and **G** (populations 5, 6 and 7) – resistant x resistant crosses. The
 695 linear regression models describe the dependence of plant wilt on vascular
 696 discoloration length. The coefficient of determination, R^2 describes the proportion of
 697 variance of plant wilting explained by the vascular discoloration length. The P -value
 698 indicates whether the regression model was significant.

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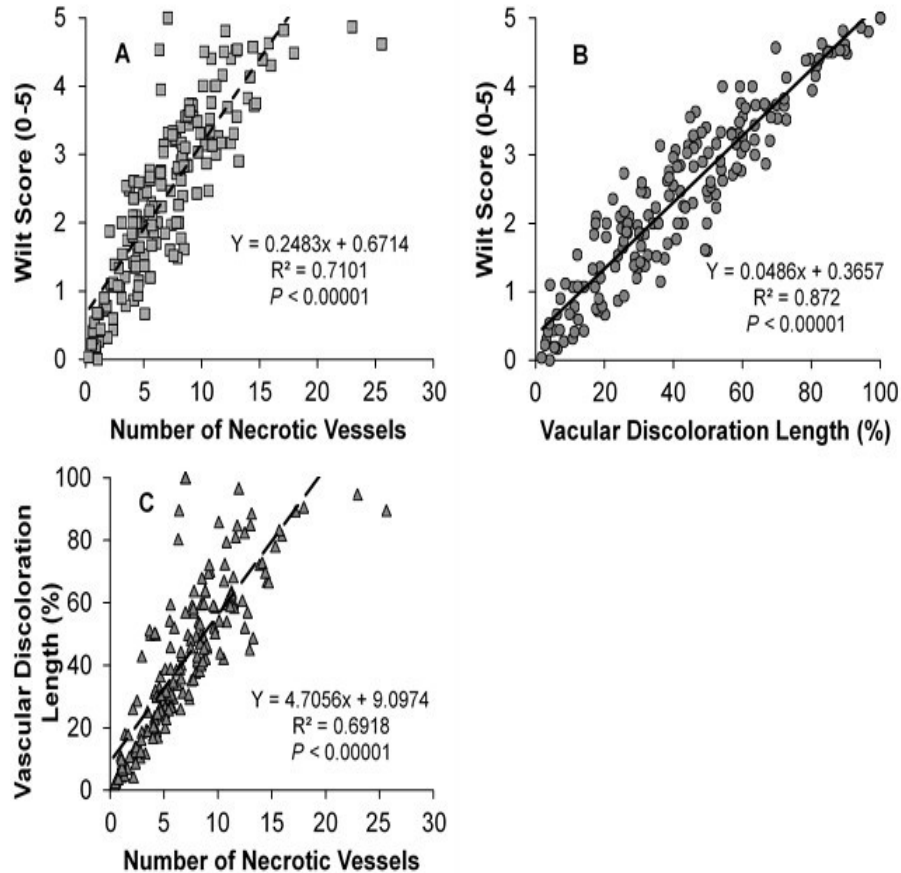
702

703 **S2.** Relationship between vascular discoloration length and number of necrotic
704 vessels in seven F_2 populations (A, B, C, D, E, F and G). **A, B, C** and **D** (populations
705 1, 2, 3 and 4) – susceptible x resistant crosses; **E, F** and **G** (populations 5, 6 and 7) –
706 resistant x resistant crosses. The linear regression models describe the relationship
707 between vascular discoloration length and the number of necrotic vessels. The
708 coefficient of determination, R^2 describes the proportion of variance of necrotic
709 vessels explained by the vascular discoloration length. The P -value indicates
710 whether the regression model was significant.

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715 **S3. A and B:** The relationship between wilt score and the number of necrotic vessels
716 and vascular discoloration, respectively in the $F_{2:3}$ population (population 8). **C:** the
717 relationship between vascular discoloration and the number of necrotic vessels in the
718 $F_{2:3}$ population. The linear regression models are represented by the curve and
719 equation. The coefficient of determination, R^2 explains the proportion of variance of
720 plant wilting explained by **A:** number of necrotic vessels and **B:** vascular
721 discoloration length; and **C:** proportion of variance explained by the relationship
722 between both metrics measures to describe vascular phenotypes. The P -value
723 indicates whether the regression model was significant.