Uncovering *Fusarium* species diversity, distribution and pathogenicity associated with cotton and corn fields in recently expanded cotton-growing areas of the Northern High Plains of Texas

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Keywords: *Gossypium* species, maize, *Zea mays*, Texas High Plains, *Fusarium* diseases, *Fusarium* diversity, *Fusarium* wilt, *Fusarium oxysporum* f. sp. *vasinfectum*, arid climate

Funding: Cotton Inc. 24-115

Texas A&M AgriLife Research Capacity Support

Abstract

The recent expansion of cotton acreage in the Northern High Plains of Texas has raised concerns about the occurrence of a diverse *Fusarium* community, particularly *Fusarium oxysporum* f. sp. *vasinfectum* race 4, which is favored by cool early-season temperatures that often occur in the region. This study investigated the diversity, distribution, and pathogenicity of *Fusarium* species associated with cotton and corn fields across 10 counties in the Northern High Plains. A total of nine *Fusarium* species were isolated: *F. incarnatum*, *F. equiseti*, *F. solani*, *F. verticillioides*, *F. proliferatum*, *F. clavum*, *F. oxysporum*, *F. flocciferum*, and one unidentified *Fusarium* sp. The highest species diversity was observed in Sherman County. *Fusarium solani* was the most frequently isolated species (39%) across all counties, followed by *F. equiseti* (22%), while *F. flocciferum* was the least frequent (2%). Pathogenicity assays conducted under greenhouse conditions showed that *F. incarnatum* caused significantly higher disease severity on aerial parts of cotton, while *F. flocciferum* and *F. proliferatum* caused significantly higher disease severity on roots. On corn, *F. proliferatum* caused significantly higher disease severity. *Fusarium* isolates were recovered from both symptomatic and asymptomatic roots following pathogenicity trials, suggesting endophytic colonization. This study revealed a complex *Fusarium* community capable of cross-infecting cotton and corn, with implications for regional disease management. The absence of *Fusarium oxysporum* f. sp. *vasinfectum* race 4 indicates that it may not yet have been introduced into the Northern High Plains; however, continued surveillance is warranted given its detection in New Mexico and adjacent counties in Texas.

The Northern High Plains are a mixture of rangeland and cropland, comprising the 22 northernmost counties in Texas (Zivkovic and Hudson 2012). The region faces severe water scarcity, with an annual mean precipitation of about 558 mm–well below the US national average of 965 mm per year (BestPlaces 2025; NOAA 2021). This water scarcity was especially pronounced during the 2019 drought, which led to crop failure on over 70% of the regional acreage, marking one of the worst cotton production seasons since the 1950s (Taylor 2022). The region relies heavily on water drawn from the Ogallala Aquifer to produce irrigated crops. However, current water use is unsustainable, with annual extraction exceeding annual recharge (Thayer and McCarl 2018). As a result, growers are increasingly shifting from high water-demand crops, such as wheat, to less water-intensive alternatives like cotton (Colaizzi et al. 2009). Temperatures in the Northern Panhandle are lower than in other parts of Texas, with mean annual temperature of about 11°C (Kimmel Jr et al. 2016). Average January low temperatures range from -6.6°C along the northern border of the Panhandle to 10°C in the Lower Rio Grande Valley (Kimmel Jr et al. 2016).

Approximately 7 million beef cattle are raised annually in beef cattle feedyards in the Texas High Plains (TCFA 2015). As of March 2025, there were 2.35 million head of cattle and calves on feed in the Northern High Plains, accounting for 89 percent of the state's total (USDA National Agricultural Statistics Service 2025). Corn remains the primary feed crop, with more than 50 percent of the corn grown in Texas coming from the High Plains region (National IPM Database 2001; Xue et al. 2017). Although corn is the major feed crop, there has been significant interest in growing cotton as a rotational

66 crop due to water constraints and the high fertilizer costs associated with corn
67 production (Colaizzi et al. 2009).
68 Cotton (*Gossypium* species) is the most important fiber crop for the textile
69 industry and a significant oilseed crop globally. The United States is the world's fourth-
70 largest cotton producing country (USDA Foreign Agricultural Service 2024), with
71 cultivation spanning 17 southern states. Texas is the leading producer, accounting for
72 approximately 40% of the U.S. cotton production in recent years (USDA Economic
73 Research Service 2022). Although cotton was introduced to the Northern High Plains in
74 1968, acreage remained below 20,000 ha until 1998, but has expanded substantially
75 since 2004—including a 126% increase between 2013 and 2019—raising concerns
76 about diseases that may affect cotton production (Ledbetter 2020; Marek and
77 Bordovsky 2006).
78 Key fungal genera implicated in cotton diseases worldwide and in the U.S. Cotton
79 Belt include *Fusarium*, *Verticillium*, *Rhizoctonia*, and *Pythium*. Among these, *Fusarium*,
80 *Rhizoctonia*, and *Pythium* species are primarily associated with the seedling disease
81 complex (Howell 2002; Watkins 1981), while *Fusarium* species—alone or in
82 combination with *Alternaria*, *Aspergillus* species, or bacterial pathogens such as
83 *Pantoea agglomerans* and *Serratia marcescens*—are frequently linked to seed rot
84 (Glover et al. 2020; Medrano et al. 2007; Minton and Garber 1983). Diseases caused
85 by oomycetes and splash dispersed pathogens are not considered a major concern in
86 the Northern High Plains due to the dry climate and severe water scarcity.
87 Fusarium wilt, caused by *Fusarium oxysporum* f.sp. *vasinfectum* (FOV), is of
88 particular concern in the Northern High Plains because cooler temperatures during the

early planting season favor its development (Zhang et al. 2020). FOV was first detected in the United States in 1892 (Atkinson 1892). Historically, FOV has been characterized into 8 different races based on variation in pathogenicity to various plant species, including *Gossypium* species and cultivars. Among these, race 4 (FOV4) is of particular concern in the U.S. due to its high virulence on both Upland and Pima cotton, as well as its ability to persist in soil for years as chlamydospores (Hutmacher et al. 2013; Kim et al. 2005). Additionally, unlike other races, FOV4 can cause severe wilting in the absence of root knot nematode *Meloidogyne incognita.* FOV4 was first detected in the U.S. in 2001 in California (Kim et al. 2005) and more recently in 2017 in Texas (Halpern et al. 2018) and 2019 in New Mexico (Zhu et al. 2020). This highly aggressive race has not yet been reported in the Northern High Plains. However, given the proximity to New Mexico and adjacent counties in Texas where FOV4 has been detected, there is great concern about its potential introduction into the Northern High Plains. Therefore, a comprehensive survey is urgently needed to investigate the presence or absence of FOV4 in the region.

Several surveys have been conducted across the U.S. Cotton Belt to determine the diversity, distribution, and severity of *Fusarium* species (Davis et al. 2006; Ilango and Uthamasamy 1992; Zaki et al. 2021). However, these surveys did not include the Northern High Plains, leaving limited information on the diversity, distribution and relative severity of *Fusarium* species affecting cotton in this region. Moreover, these studies were primarily focused on investigating the presence or absence of FOV4 rather than determining overall *Fusarium* species diversity. Several *Fusarium* species are known to cause economically important diseases in cotton, including wilt, damping-off, boll rot,

112 and root rot (Davis et al. 2006; Ilango and Uthamasamy 1992; Zaki et al. 2021). While
113 *F. oxysporum* and *F. solani* are the most common species complexes associated with
114 cotton, the relative severity and impact of other *Fusarium* species remain poorly
115 understood (Mao and Hu 2019).

116 Crop rotation has been recognized as an effective practice to reduce nitrogen
117 fertilizer use and enhance both grain yield and sustainability in agricultural systems (Shu
118 et al. 2021; Zhang et al. 2022). However, crop rotation can also inadvertently increase
119 inoculum levels, thereby elevating the risk of crop diseases (Li et al. 2021). Previous
120 studies have demonstrated that crop residue serves as a natural reservoir for *Fusarium*
121 species and may even facilitate genetic exchange (Ward et al. 2008). For instance, in
122 wheat–corn cropping rotations, continuous wheat cultivation following corn has been
123 shown to increase the incidence of *Fusarium* stalk rot and ear rot in maize, as well as
124 *Fusarium* head blight in wheat (Xi et al. 2021; Zhang et al. 2016). In the Northern High
125 Plains, where cotton and corn are commonly grown in rotation, investigating the
126 pathogenicity of *Fusarium* species on both crops is crucial. Such studies will provide
127 valuable insights into the pathogen's persistence, cross-infectivity, and the associated
128 epidemiological risks.

129 Greenhouse pathogenicity assays offer several advantages, including reduced
130 variability resulting from uneven inoculum distribution and external biotic or abiotic
131 factors. Most pathogenicity assays conducted in greenhouse conditions use root-dip
132 inoculation method, where two-week-old seedlings' roots are dipped in a spore
133 suspension for two minutes and then transplanted into healthy soil (Kim et al. 2005).
134 This method can predispose seedlings to stress during transplantation and does not

135 reflect host infection in the field (Schoeneweiss 1975). Conversely, pathogenicity assays
136 that involve directly planting seeds in *Fusarium*-infested soil or adding inoculum after
137 seedling emergence better reflect infection under field conditions.

138 To address these key gaps, the present study was conducted to: (1) conduct a
139 comprehensive survey to determine the diversity and distribution of *Fusarium* species
140 associated with cotton and corn fields in the recently expanded cotton-growing areas of
141 the Northern High Plains; (2) investigate the presence or absence of FOV4 in the region;
142 and (3) evaluate the pathogenicity and severity of *Fusarium* species on cotton and corn
143 under greenhouse conditions.

144 Materials and Methods

145 *Sampling sites and sampling procedure*

146 With the help of cotton growers and county agents, a total of 26 cotton fields
147 across 10 different counties in the Northern High Plains of Texas were sampled between
148 late spring and late fall (May to October) of 2024. Specifically, six fields were sampled in
149 Sherman County; four fields each in Moore and Deaf Smith counties; three fields in
150 Hartley County; two fields each in Randall, Hansford, and Carson counties; and one field
151 each in Potter, Dallam, and Hutchinson counties.

152 In each field, a total of 5 soil cores, each 0.2 m deep, were collected at 5 different
153 locations, with at least 60 m space between sampling points. The soil samples from
154 each field were pooled and placed into cloth bags, kept on ice in an insulated box to
155 protect them from high temperature and direct sunlight, and carried to the laboratory.

156 In the laboratory, the samples collected from each field were mixed thoroughly
157 and air dried for a week. The air-dried soil sample for each field was poured into a

158 Nasco-Asplin soil grinder, pulverized gently to break the clods, and passed through a 2
159 mm sieve. To prevent cross-contamination between samples, the excess dirt from
160 grinder's parts were removed with a brush, washed with water, and then cleaned
161 thoroughly with 70% ethanol and paper towels.

162 Diversity and distribution of *Fusarium* species in the Northern High Plains

163 *Isolation of single spore isolates from soil*

164 To isolate *Fusarium* species from soil, a total of 20 cc of soil was added to 80 mL
165 of reverse osmosis (RO) water in a small beaker (200 mL) placed on a magnetic stirrer
166 and mixed vigorously to ensure thorough mixing of the soil. From this soil suspension, a
167 1 mL aliquot was plated onto each of 5 Petri dishes of Peptone PCNB agar (PPA)
168 medium (1000 mL distilled water, 15 g Peptone, 1 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.75 g
169 PCNB, 20 g agar, pH adjusted to 5.5–6.5) (Nash and Snyder 1962). The process was
170 repeated with an additional 20 cc of soil using another set of five Petri dishes, allowing
171 us to assess 40 cc of soil for each field using a total of 10 Petri dishes. The Petri dishes
172 were incubated for 3 days at room temperature (23 ± 2°C) with a 12-hour photoperiod.
173 The colonies developed on PPA were subcultured onto potato dextrose agar (PDA)
174 medium (1000 mL distilled water, 39 g PDA agar) by taking small mycelial plugs,
175 observed under a Leica M205C stereoscope at 160x magnification, from the colony
176 margins.

177 After incubating for two weeks, 200 µL of RO water was added to each Petri dish,
178 and conidia were dislodged by gently scrapping the mycelium with a pipette tip. A total of
179 150 µL of this conidial suspension was then transferred onto water agar (30 g agar,
180 1000 mL RO water) and incubated overnight. Purified cultures were obtained by

181 transferring single germinated conidia, observed under Nikon SM225 microscope at
182 180x magnification, onto fresh PDA plates.

183 *Genomic DNA extraction, PCR amplification, and sequencing*

184 Isolates were divided into morphotypes based on their gross colony morphology.
185 Two to three isolates from each morphotype were selected for sequence-based
186 identification using the translation elongation factor 1-α (tef-1α) gene region for all
187 isolates and the nuclear ribosomal internal transcribed spacer region (ITS rDNA) for five
188 isolates (Table 1). DNA was extracted using Zymo Quick DNA Fungal/Bacterial
189 Microprep kit (Zymo Research Corp; Irvine, CA). Amplicons were obtained using the
190 following primer pairs:EF-1 (5′-ATGGGT AAGGARGACAAGAC-3′) × EF-2 (5′-
191 GGARGTACCA GTSATCATG-3′) for tef-1α gene region (O'Donnell et al. 1998); and
192 ITS1 (TCCGTAGGTGAACCTGCG) × ITS4 (TCCTCCGCTTATTGATATGC) for ITS
193 rDNA (White et al. 1990).

194 The PCR reaction mixture (25 µl reaction) contained 12.5 µl of Phire Green Hot
195 Start II Master Mix (Thermo scientific), 1.25 µl of each primer (0. 5 µM), and 1.5 µl of
196 genomic DNA. The PCR amplification program conditions were as follows: initial
197 denaturation at 98°C for 90 s followed by 35 cycles at 98°C for 5 s, annealing at 58°C for
198 15 s and extension at 72°C for 15 s, followed by final extension for 4 minutes at 72°C.

199 The PCR products were detected using 1% agarose gel in 1x TAE buffer. The
200 PCR products were cleaned using DNA Clean & Concentrator kit (Zymo Research) and
201 sent to Eurofins Genomics (Eurofins Genomics LLC, Louisville, Kentucky) for
202 sequencing with both forward and reverse primers. ABI sequence chromatograms were
203 edited using SnapGene 8.0 (https://www.snapgene.com/) and exported as FASTA files

204 that were aligned using MUSCLE alignment (Edgar 2004) in Mega11
205 (https://www.megasoftware.net/). Consensus sequences were obtained using BioEdit
206 7.7.1 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html), which were then used to conduct
207 BLASTn queries of NCBI GenBank (https://www.ncbi.nlm.nih. gov/genbank/) to obtain
208 preliminary identifications.

209 Given the regional importance of *F. oxysporum*, the isolates identified as *F.*
210 *oxysporum* were additionally amplified using RNA polymerase II second largest subunit
211 (RPB2) (Liu et al. 1999; O'Donnell et al. 1998; O'Donnell et al. 2000; Simko and
212 Piepho 2012) with the primer pair: 5f2 (GGGGWGAYC AGAAGAAGGC) × 7cr
213 (CCCATRGCTTGYTTRCCCAT), using the PCR conditions described above.

214 *Phylogenetic analyses*

215 Nucleotide sequences generated in this study, along with reference sequences
216 retrieved from the NCBI GenBank database, were aligned using MUSCLE alignment
217 (Edgar 2004) in Mega11 (https://www.megasoftware.net/). Alignments were then
218 trimmed avoiding missing data at either end of alignments. The aligned sequences were
219 analysed with maximum likelihood bootstrapping (MLBS) using IQ-TREE (http://
220 www.iqtree.org/) (Nguyen et al. 2015) to determine the best-fit model of molecular
221 evolution. These models were based on the Bayesian information criterion (BIC) scores
222 (Chernomor et al. 2016) using ModelFinder (Kalyaanamoorthy et al. 2017). Once the
223 optimal models were determined, clade support was assessed via 50,000 ML-BS
224 pseudoreplicates of the data using IQ-TREE.

225 To determine the phylogenetic placement of *Fusarium oxysporum* sequences
226 generated in this study relative to known FOV races (Cianchetta et al. 2015) and closely

227 related sequences, additional reference sequences representing all recognized FOV
228 races and top BLASTn hits from the NCBI GenBank database were retrieved and
229 phylogenetic analysis was conducted as described above. DNA sequences generated in
230 the present study were deposited in NCBI (Table 1).

231 Pathogenicity trials

232 Seven isolates, each representing a different *Fusarium* species, were selected to
233 test their pathogenicity and disease severity on wheat and cotton. These included *F.*
234 *incarnatum, F. equiseti, F. solani, F. verticillioides, F. proliferatum, F. clavum, and F.*
235 *flocciferum.* Given its regional importance, *F. oxysporum* was not included in the
236 inoculated pathogenicity trials.

237 *Pathogenicity of Fusarium species on cotton*

238 *Biological material and experimental design*

239 The trial was conducted under greenhouse conditions at Texas A&M AgriLife
240 Research, Bushland, Texas at ambient temperature (25 ± 5°C). A commonly cultivated
241 variety in the Texas High Plains, FiberMax 868AXTP, with fair resistance to *Fusarium*
242 wilt was used in this study (BASF 2024). Disease-free seeds of this variety were sourced
243 from a previous trial: seeds were de-linted but received no chemical treatment prior to
244 planting. Briefly, 10-cm pots, serving as experimental units, were filled with a modified
245 media (1 bag of Berger's OM1 potting mix, 0.085 m^3 of organic growing mix, 2.2 kg of
246 sand, and 3 kg of soil). A total of 2-3 disease-free seeds were sown in each pot. Upon
247 germination, seedlings were thinned to one per pot. There were six replicate pots for
248 each *Fusarium* species and a non-inoculated control (total 48 plants), arranged in a

249 completely randomized block design. Throughout the trial period, seedlings were
250 fertilized with water-soluble fertilizer 'Miracle-Gro' as needed.

251 *Inoculum preparation*

252 The sorghum grain inoculation method was used to prepare the inoculum.
253 Disease-free sorghum grains were sourced from a previous year's trial. Briefly, sorghum
254 grains were soaked overnight, and 50 g of soaked grains were placed into each 250 mL
255 beaker and sealed with aluminium foil. The beakers were autoclaved at 121°C for 30
256 minutes on two consecutive days. Once cooled, the sorghum grains in each beaker
257 were inoculated with five agar plugs (5 mm), which were cut from a 7-day-old colony of
258 specific *Fusarium* isolate grown on PDA. The inoculated beakers were gently shaken to
259 uniformly distribute the inoculum and then placed inside zip-lock plastic bags to avoid
260 contamination. The inoculated beakers were incubated for 7 days at room temperature
261 (23 ± 2°C) with a 12-hour photoperiod and shaken every 2–3 days to ensure even
262 distribution of the inoculum.

263 *Inoculation method*

264 Cotton seedlings were inoculated at 2–3 leaf stage. Seedlings were watered
265 thoroughly in the evening prior to inoculation the following morning. To inoculate
266 seedlings, four 5-cm deep holes were made vertically into the potting medium of each
267 pot using a wooden rod (18 x 0.8 cm), approximately 2 to 4 cm from the crown of each
268 plant. A total of 1.25 g inoculum was inserted into each hole, excluding the non-
269 inoculated controls. The holes were then covered with potting medium, and plants were
270 watered daily following inoculation.

Disease assessment

Cotton seedlings were observed daily for the appearance of disease symptoms. Disease severity on aerial plant parts was recorded at 7, 14, 21, and 28 days post-inoculation using a 0–4 rating scale, where: 0 = healthy; 1 = cotyledons wilted only; 2 = ≤50% true leaves wilted; 3= >50% but ≤90% true leaves wilted; and 4 = all leaves wilted or plant death (Table S1).

All cotton seedlings were harvested after one month of inoculation. Roots were washed free of soil and disease severity was recorded using 0–5 rating scale, where 0 = no symptoms; 1 = <10% of roots discolored; 2 = 10–30% of roots discolored; 3= 31–50% of roots discolored; 4 = 51–80% of roots discolored; and 5 = 81–100% of roots discolored (Table S1).

At the end of the trial, the roots of all cotton seedlings were plated onto PDA (1000 mL RO water, 39 g potato dextrose agar, 1 g streptomycin) to perform Koch's postulates.

Pathogenicity of Fusarium species on corn

Biological material, experimental design, inoculum preparation and inoculation method

A commonly cultivated drought tolerant, untreated corn hybrid, Blue River Organic 82-14P, was used in the pathogenicity trial.

The experimental design, inoculum preparation and application method, as well as cultural practices, were the same as those used in the cotton pathogenicity trial. That is, a single batch of inoculum was used to inoculate both cotton and corn seedlings at the same time and similar cultural practices were followed to ensure consistency across experiments. Seedlings were inoculated at the three-leaf stage.

294 *Disease assessment*

295 Corn seedlings were harvested after one month of inoculation. Roots were
296 washed free of soil and crown and root lesions were rated using a 1–8 rating scale
297 (Parikh et al. 2018), where: 1 = no visible symptoms, 0% infection, and normal
298 development; 2 = small speck of lesions near the crown and/or superficial root infection.
299 Infection is <10%, and development is normal; 3 = superficial lesions visible near the
300 crown or stem. Infection is 10%–20%, and development is normal; 4 = deep lesion has
301 advanced from crown and lower roots to the middle stem. Infection is 21%–40%, and
302 development is normal; 5 = deep lesion visible through approximately half the length of
303 the stem and root. Infection is 40%–60%, and plant development is affected; 6 = deep
304 lesions on most parts of the stem and root, reduced root growth, significantly reduced
305 plant development, and stunting are observed. Infection is 60%–85%; 7 = severe deep
306 lesions covering almost all parts of the stem and root, significantly reduced root growth
307 and plant development, and extensive stunting are observed. Infection is 85%–95%; 8 =
308 seedling barely emerged and could not grow, scanty root but no emergence, or seed is
309 dead (no emergence and no roots). Infection coverage of seedling is >95% (Table S1).

310 At the end of the trial, the roots of all corn seedlings were plated onto PDA (1000
311 mL RO water, 39 g potato dextrose agar, 1 g streptomycin) to confirm Koch's postulates.

312 Data Analysis

313 In the case of disease severity recorded on aerial parts of cotton, the area under
314 the disease progress stairs (AUDPS) was calculated using the 'audps()' function from
315 'agricolae' R package (Mendiburu 2023), based on severity ratings recorded across all
316 four disease assessments. The AUDPS was selected over the traditional area under the

317 disease progress curve (AUDPC) because the latter underestimates the effect of
318 disease severity recorded during first and last disease assessments (Simko and Piepho
319 2012). Linear mixed models effect models (LMMs) were then fitted using the 'lmer()'
320 function from the 'lmerTest' R package (Kuznetsova et al. 2022) to investigate
321 differences in disease severity among *Fusarium* species. The AUDPS was included as
322 the dependent variable, the predictor *Fusarium* species were included as a fixed effect,
323 and different blocks were included as a random effect to account for variability across
324 blocks.

325 In the case of disease severity recorded on roots of cotton and corn, disease
326 ratings were converted to the midpoint of the corresponding percentage range (Table
327 S1). These mid-points were then converted to proportions and subsequently logit
328 transformed following a preliminary analysis indicating that linear model's residuals were
329 not normally distributed. For cotton disease rating data, zero disease severity ratings
330 were converted 0.001 to allow logit transformation. The differences in disease severity
331 among *Fusarium* species were then determined by fitting LMMs. The logit transformed
332 midpoint proportions were included as the dependent variable, the predictor *Fusarium*
333 species were included as a fixed effect, and different blocks were included as a random
334 effect to account for variability across blocks.

335 For all models, estimated marginal means for each *Fusarium* species were
336 calculated using the 'emmeans()' function from 'emmeans' R package (Lenth 2021). The
337 estimated marginal means were compared using Tukey-adjusted pairwise comparisons,
338 and groupings were assigned using the 'cld()' function from the 'multcomp' R package
339 (Hothorn 2020). These groupings indicated significant differences at $\alpha = 0.05$. Models

340 diagnostics was performed using the 'DHARMa' R package, which uses a simulation-
341 based approach via the function 'simulateResiduals' to create readily interpretable
342 scaled residuals for models (Hartig 2020).

343 ## Results

344 ### *Diversity and distribution of Fusarium species in the Northern High Plains*

345 A total of nine *Fusarium* species were identified: these included *F. incarnatum, F.*
346 *equiseti, F. solani, F. verticillioides, F. proliferatum, F. clavum, F. oxysporum, F.*
347 *flocciferum,* and *Fusarium* sp. (Table 1, Figure 1). Colony morphology on PDA varied
348 among isolates belonging to the same species, particularly for *F. solani.* No PCR
349 amplifications were achieved for *F. oxysporum* isolates identified in this study using
350 FOV4 specific primers. Phylogenetic analysis of tef-1α gene region placed *F. oxysporum*
351 isolate F17 within a clade containing reference sequence of *F. oxysporum* f.
352 sp. *asparagi* , while the isolate F34 clustered with the reference sequence of *F.*
353 *oxysporum* f. sp. *lactucae* (Figure S1). *Fusarium solani* was the most frequently isolated
354 species (39%), followed by *F. equiseti* (22%), *F. proliferatum* and *F. clavum* (7% each),
355 *F. incarnatum*, *F. verticillioides*, *F. oxysporum*, and *Fusarium* sp. (5% each), and *F.*
356 *flocciferum* (2%) (Table 1, Figure 2A).

357 Although no single county harbored all species isolated in this study, five species
358 were recovered from Sherman County, including *F. incarnatum*, *F. equiseti*, *F. solani*, *F.*
359 *verticillioides*, and *Fusarium* sp. (Table 1). This was followed by Moore County (*F.*
360 *incarnatum*, *F. equiseti*, *F. solani*, and *F. proliferatum*) and Carson County (*F. equiseti*,
361 *F. solani*, *F. oxysporum*, and *F. flocciferum*), each with four species isolated (Table 1).
362 Two species were restricted to a single county. That is, *F. verticillioides* was isolated

363 only from Sherman County, while *F. flocciferum* was isolated from Carson County only
364 (Table 1).

365 *Pathogenicity of Fusarium species on cotton*

366 All *Fusarium* isolates tested were pathogenic on cotton, causing typical symptoms
367 associated with *Fusarium* infections (Figure 3). No disease was recorded on non-
368 inoculated control plants. *Fusarium* isolates were re-isolated from all inoculated roots,
369 both symptomatic and asymptomatic, plated on PDA (i.e., 100% re-isolation rate).

370 For the aerial parts, the highest disease severity (expressed as AUDPS) was
371 caused by *F. incarnatum* (31.4), followed by *F. solani* (12.5), while the lowest disease
372 severity was associated with *F. verticillioides* (7.1) (Figure 4A). Only *F. incarnatum*
373 caused significantly higher disease severity relative to non-inoculated control (Figure 4B,
374 P=0.0137, EMMeans pairwise comparisons). Disease severity associated other
375 *Fusarium* species did not differ significantly from the non-inoculated control or from each
376 other (Figure 4B, P>0.05, EMMeans pairwise comparisons).

377 For roots, the highest disease severity, expressed as mean proportion of
378 midpoints, was caused by *Fusarium flocciferum* (0.31) and *F. proliferatum* (0.26),
379 whereas the lowest disease severity was caused by *F. incarnatum* (0.08) and *F. equiseti*
380 (0.05) (Figure 2B). *Fusarium flocciferum*, *F. proliferatum, F. clavum* and *F. solani* caused
381 significantly higher disease severity relative to non-inoculated control (Figure 5A,
382 P=0.0011, P=0.0013, P=0.0136, P=0.0461, respectively; EMMeans pairwise
383 comparisons). Conversely, disease severity caused by *F. equiseti, F. incarnatum, and F.*
384 *verticillioides* did not differ significantly from the non-inoculated control (Figure 5A,
385 P>0.05, EMMeans pairwise comparisons). No significant differences in disease severity

386 were recorded among different *Fusarium* species (Figure 5A, *P*>0.05, EMMeans
387 pairwise comparisons). DHARMa diagnostics showed that the data met the model
388 assumptions.

389 *Pathogenicity of Fusarium species on corn*

390 All *Fusarium* isolates tested were pathogenic on corn. The highest disease
391 severity, expressed as mean proportion of midpoints, was caused by *F. proliferatum*
392 (0.12), *F.* flocciferum and *F. verticillioides* (0.05), while the lowest disease severity was
393 associated with *F. solani* (0.0083) (Figure 2B). No disease was recorded on non-
394 inoculated control plants. Like cotton, *Fusarium* species were re-isolated from all
395 inoculated corn plants, regardless of whether they exhibited symptoms.

396 *Only F. proliferatum* caused significantly higher disease severity relative to both *F.*
397 *solani* and non-inoculated control (Figure 5B, *P*=0.0407 and *P*=0.0076, respectively;
398 EMMeans pairwise comparisons). *Fusarium proliferatum, F. flocciferum* and *F.*
399 *verticillioides* caused significantly higher disease severity from the non-inoculated control
400 plants only (Figure 5B, *P*=0.0076, *P*=0.0279, and *P*=0.0279, respectively; EMMeans
401 pairwise comparisons). Except for *F. solani* and *F. proliferatum,* no significant
402 differences in disease severity were recorded among *Fusarium* species (Figure 5B,
403 *P*>0.05, EMMeans pairwise comparisons). DHARMa diagnostics showed that the data
404 met the model assumptions.

405 Discussion

406 To the best of our knowledge, this study is the first to conduct a comprehensive
407 survey of the recently expanded cotton-growing areas in the Northern High Plains of
408 Texas and provided valuable insights into the diversity, distribution, and pathogenicity

409 of *Fusarium* species associated with cotton and corn fields. A total of nine *Fusarium*
410 species were identified, with *F. solani* being the most frequently isolated and *F.*
411 *flocciferum* the least. The diversity and distribution of *Fusarium* species varied across
412 counties, with Sherman County harbouring the highest number of *Fusarium* species. All
413 tested species were pathogenic on cotton and corn during the pathogenicity assays
414 conducted under greenhouse conditions.

415 The high frequency of *F. solani* and *F. equiseti* isolation in the present study
416 aligns with prior findings from Arizona and Alabama, where they were the most
417 frequently isolated species from cotton roots and rhizosphere soil (Mao and Hu 2019;
418 Palmateer et al. 2002). Likewise, *F. solani* was reported as the most predominant
419 species, isolated from 97% of sorghum and corn fields across central and southeastern
420 United States (Leslie et al. 1990). Conversely, the less frequent isolation of *F.*
421 *flocciferum* in the present study is consistent with the findings from previous studies
422 conducted in France (Nguyen et al. 2024) and Belgium (Scauflaire et al. 2011), where it
423 was among the least frequently isolated species in corn agroecosystems. To date, there
424 are no reports of *F. flocciferum* as a widely distributed pathogen in the U.S. Cotton Belt.
425 These findings suggest that *F. solani* and *F. equiseti* are habitat generalists, being
426 widely distributed across the U.S. Cotton Belt, whereas *F. flocciferum* is mostly a habitat
427 specialist with a narrow distribution range.

428 The observation that colony morphology on PDA varied among isolates of the
429 same species, particularly *F. solani,* is consist with finding from a previous study (Mao
430 and Hu 2019) and suggests that morphological characteristics alone are insufficient for
431 species identification, emphasizing the need for sequence-based identification.

432 The variation in *Fusarium* species diversity observed across counties in this study
433 is likely caused by a combination of factors, including environmental conditions, soil
434 types, cultural practices (e.g., crop rotation and tillage), and predisposing factors such as
435 the presence of nematodes or flooding events. Differences in cotton acreage among
436 counties may have also contributed to variation in species diversity across counties.
437 Specifically, the highest species diversity in Sherman, Moore, and Carson counties can
438 partly be attributed to the substantial increase in cotton acreage between 2013 and
439 2018, during which production grew by 301% to over 250,000 acres (Ledbetter 2020;
440 Marek and Bordovsky 2006).

441 The significantly higher disease severity observed in cotton inoculated with *F.*
442 *incarnatum*, *F. flocciferum*, *F. proliferatum*, *F. clavum*, and *F. solani*, as well as in corn
443 inoculated with *F. proliferatum*, *F. verticillioides*, and *F. flocciferum* (relative to non-
444 inoculated control plants in both crops), suggests that these species may pose a
445 potential threat to cotton and corn production in the Northern High Plains. Among these,
446 *F. proliferatum* and *F. solani* have been previously identified as the most virulent species
447 on cotton (Abdel-Latif et al. 2007; Palmateer et al. 2002), whereas *F. proliferatum* and
448 *F. verticillioides* have been described as the most virulent species on corn (Okello et al.
449 2019; Wang et al. 2021). The pathogenicity of *Fusarium* species on both cotton and
450 corn suggests that they can overwinter on the stubble of either crop and serve as a
451 primary source of inoculum in the next spring.

452 However, overall disease severity on both cotton and corn was relatively low, with
453 no significant differences among *Fusarium* species on either crop, except between *F.*
454 *solani* and *F. proliferatum* in corn. On cotton, severity was largely restricted to root

455 discoloration, while in corn, severity did not exceed 20–40%. The overall low disease
456 severity may partly be attributed to the application of inoculum after seedling emergence
457 for both cotton and corn, rather than the use of a root-dip inoculation method. Although
458 the root-dip method can result in increased disease severity, it does not closely mimic
459 natural infection under field conditions and may introduce stress during transplantation,
460 potentially predisposing seedlings to infection (Diaz et al. 2021; Schoeneweiss 1975).
461 The 100% re-isolation rate of *Fusarium* species from both symptomatic and
462 asymptomatic roots following pathogenicity trials in the present study supports the
463 previous findings that systemic colonisation can occur in the absence of visible disease
464 symptoms (Leslie et al. 1990). This implies that the pathogenicity of *Fusarium* species in
465 field may need to be interpreted with caution, as *Fusarium* species may be living in
466 plants as endophytes or secondary invaders (Leslie et al. 1990). In the case of plant
467 tissues, this further underscores the importance of sampling both symptomatic and
468 asymptomatic plant tissues for accurately determining the diversity and distribution of
469 *Fusarium* species (Leslie et al. 1990). The present study likely provided an accurate
470 estimate of *Fusarium* species diversity and distribution because isolates were recovered
471 from soil samples, which minimized the bias associated with symptom-based tissue
472 sampling and isolation. Furthermore, a previous study did not find any significant
473 differences in the number of *Fusarium* species isolated from cotton roots and
474 rhizosphere soil (Mao and Hu 2019), suggesting that soil-based isolation approaches
475 can reliably capture species diversity.
476 This study provided baseline *Fusarium* species in the Northern High Plains of
477 Texas. The detection of both common (e.g., *F. solani*, *F. equiseti*) and less frequently

reported species (e.g., *F. clavum*, *F. flocciferum*) highlights the diversity of *Fusarium* species in this region. The non-detection of the highly aggressive FOV4 in present study suggests that the pathogen may not yet have been introduced into the Northern High Plains. However, given the known presence of FOV4 in nearby regions such as El Paso, Hudspeth, and New Mexico, continued surveillance remains essential. Crop rotation is unlikely to be effective since *Fusarium* species are cross-host pathogenic and the region is primarily a rangeland where mainly susceptible crops such as cotton, corn and wheat are grown for fodder. For species with broad niches, containment and treatment (such as soil solarization) would potentially be required over vast areas and therefore be impractical. Local containment may still be an option for species with restricted niches. However, the use of resistant or tolerant cultivars should be prioritized as a primary disease management strategy.

Since *Fusarium* species diversity can vary with the time of the year, future studies should aim to collect samples across multiple seasons. Additionally, including counties not sampled in the present study will contribute to a more comprehensive understanding of *Fusarium* species diversity and distribution across the Northern High Plains of Texas.

Acknowledgements

We would like to acknowledge Dennis Coker, Marcel Fischbacher, JD Ragland, Kristy Slough, Josie Strnad, and Laura Taylor for assistance in field selection, and Kristen Tatro for greenhouse technical support. The work was supported by funding from Cotton Inc. and Texas A&M AgriLife Research.

Author-Recommended Internet Resources

https://github.com/IhsanKhaliq/HighPlainsFusa

501 **Table 1** Isolate identity, collection date, county of origin, host crop history, and GenBank

502 accession numbers for *Fusarium* species isolated in this study.

Isolate	Organism	County	Previous crop	Current crop	Sampling date	GenBank Accession number		
						tef-1α	ITS	RPB2
F1	*F. incarnatum*	Sharman	Corn	Cotton	2024/08/22	PV699158		
F10	*F. incarnatum*	Moore	Corn	Cotton	2024/08/21		PV670408	
F2	*F. equiseti*	Carson	Corn	Cotton	2024/10/24		PV670407	
F14	*F. equiseti*	Carson	Corn	Cotton	2024/10/24	PV699144		
F15	*F. equiseti*	Moore	Corn	Cotton	2024/08/21	PV699145		
F18	*F. equiseti*	Hartley	Corn	Cotton	2024/09/26	PV699148		
F20	*F. equiseti*	Sharman	Corn	Cotton	2024/08/22	PV699152		
F25	*F. equiseti*	Hansford	Cotton	Wheat	2024/05/01	PV699157		
F38	*F. equiseti*	Hutchinson	Fallow	Cotton	2024/05/21	PV717850		
F40	*F. equiseti*	Sharman	Corn	Cotton	2024/08/22	PV717843		
F41	*F. equiseti*	Sharman	Corn	Cotton	2024/08/22	PV717844		
F3	*F. solani*	Deaf Smith	Fallow	Cotton	2024/10/02	PV699137		
F4	*F. solani*	Deaf Smith	Corn	Cotton	2024/10/02	PV699138		
F7	*F. solani*	Deaf Smith	Corn	Cotton	2024/10/02	PV699140		
F8	*F. solani*	Moore	Corn	Cotton	2024/08/21	PV699141		
F9	*F. solani*	Moore	Corn	Cotton	2024/08/21	PV699142		
F19	*F. solani*	Carson	Corn	Cotton	2024/10/24	PV699149		
F21	*F. solani*	Deaf Smith	Corn	Cotton	2024/10/02	PV699153		
F22	*F. solani*	Carson	Corn	Cotton	2024/10/24	PV699154		
F23	*F. solani*	Deaf Smith	Wheat	Cotton	2024/10/02	PV699155		
F30	*F. solani*	Moore	Corn	Cotton	2024/08/21	PV717836		
F31	*F. solani*	Hutchinson	Fallow	Cotton	2024/05/21	PV717837		
F32	*F. solani*	Sharman	Corn	Cotton	2024/08/22	PV717838		
F33	*F. solani*	Dallam	Corn	Cotton	2024/09/26	PV717839		
F35	*F. solani*	Randall	Corn	Cotton	2024/08/22	PV717840		
F36	*F. solani*	Hartley	Corn	Cotton	2024/09/26	PV717841		
F37	*F. solani*	Carson	Corn	Cotton	2024/10/24	PV717842		
F5	*F. verticillioides*	Sharman	Corn	Cotton	2024/08/22	PV699139		
F42	*F. verticillioides*	Sharman	Corn	Cotton	2024/08/22	PV717845		
F12	*F. proliferatum*	Randall	Corn/Sorghum	Cotton	2024/08/22		PV670409	
F16	*F. proliferatum*	Randall	Corn/Sorghum	Cotton	2024/08/22	PV699146		
F29	*F. proliferatum*	Moore	Corn	Cotton	2024/05/01	PV717848		
F13	*F. clavum*	Hansford	Cotton	Wheat	2024/05/01	PV699143		
F26	*F. clavum*	Randall	Corn/Sorghum	Cotton	2024/05/01	PV717846		
F27	*F. clavum*	Hartley	Corn	Cotton	2024/09/26	PV717835		
F17	*F. oxysporum*	Randall	Corn/Sorghum	Cotton	2024/08/22	PV699147	PV731270	PV69915
F34	*F. oxysporum*	Carson	Corn	Cotton	2024/10/24	PV717849	PV731268	PV69915

Isolate	Organism	County	Previous crop	Current crop	Sampling date	GenBank Accession number		
						tef-1α	ITS	RPB2
F39	*F. flocciferum*	Carson	Corn	Cotton	2024/10/24	PV717851		
F24	*Fusarium sp.*	Sharman	Corn	Cotton	2024/08/22	PV699156		
F28	*Fusarium sp.*	Hansford	Wheat	Cotton	2024/05/01	PV717847		

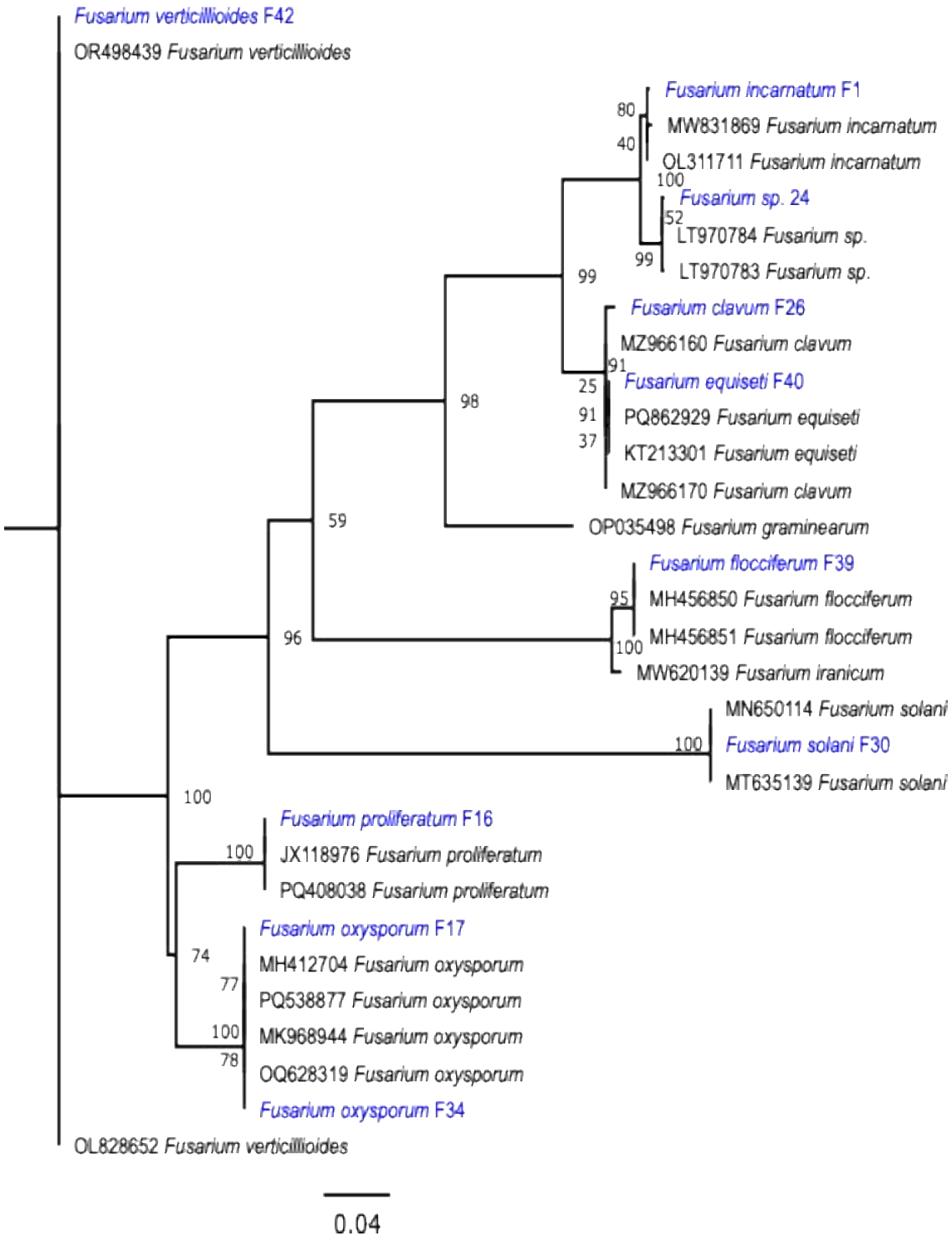

Fig. 1 Phylogenetic comparison of *Fusarium* species isolated in this study (highlighted in blue) in relation to closely related reference species based on the translation elongation factor 1-α (tef-1α) gene region.

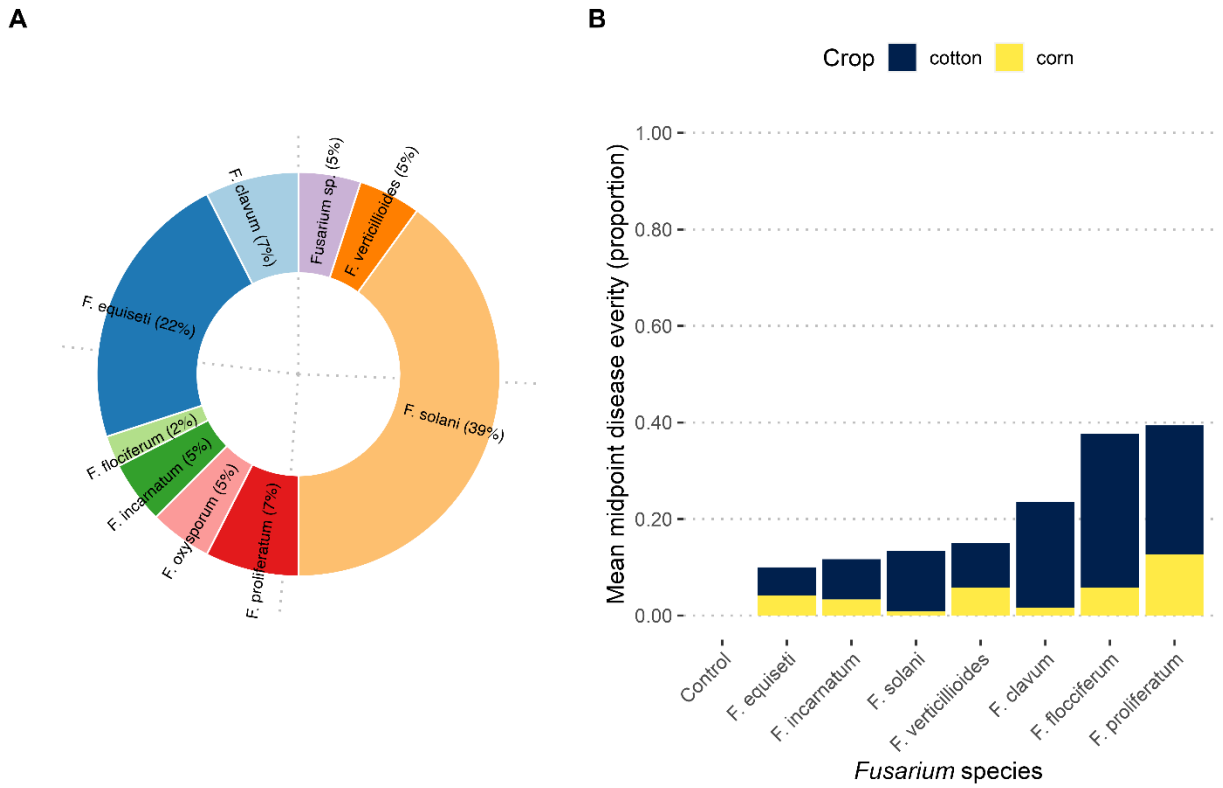

Fig. 2 (A) Frequency of Fusarium species isolated in the Northern High Plains; and (B) mean disease severity (proportion of midpoints) of *Fusarium* species on cotton and corn roots. Severity ratings were converted to the midpoint of the corresponding percentage range and then divided by 100 to obtain proportions.

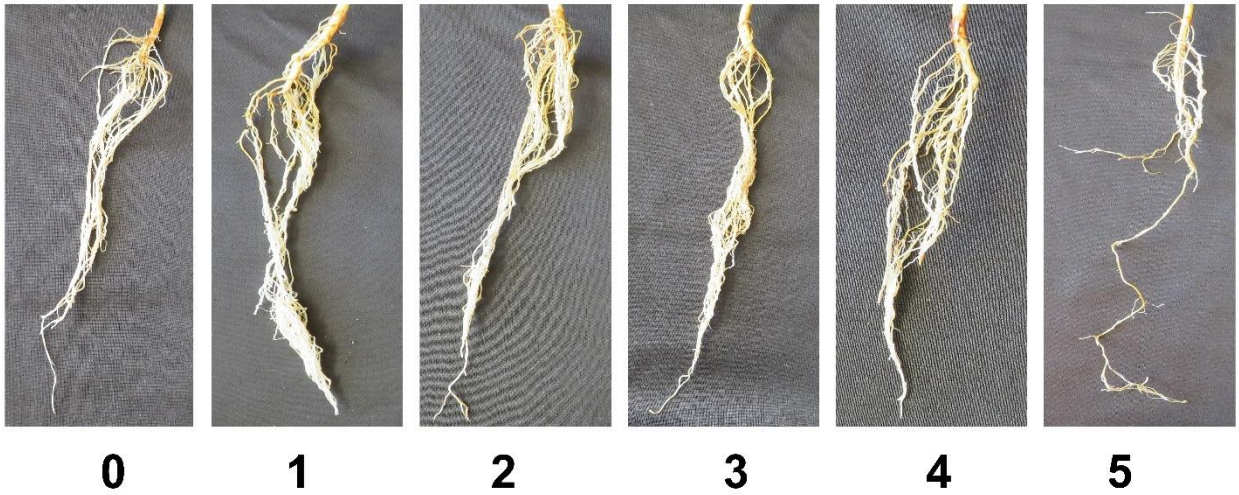

Fig. 3 Damage on roots of cotton seedlings inoculated with *Fusarium* species. Whole root systems were rated visually using a 0–5 rating scale. Left to right: 0 = no symptoms; 1 = <10% of roots discolored; 2 = 10–30% of roots discolored; 3 =31–50% of roots discolored; 4 = 51–80% of roots discolored; and 5 = 81–100% of roots discolored.

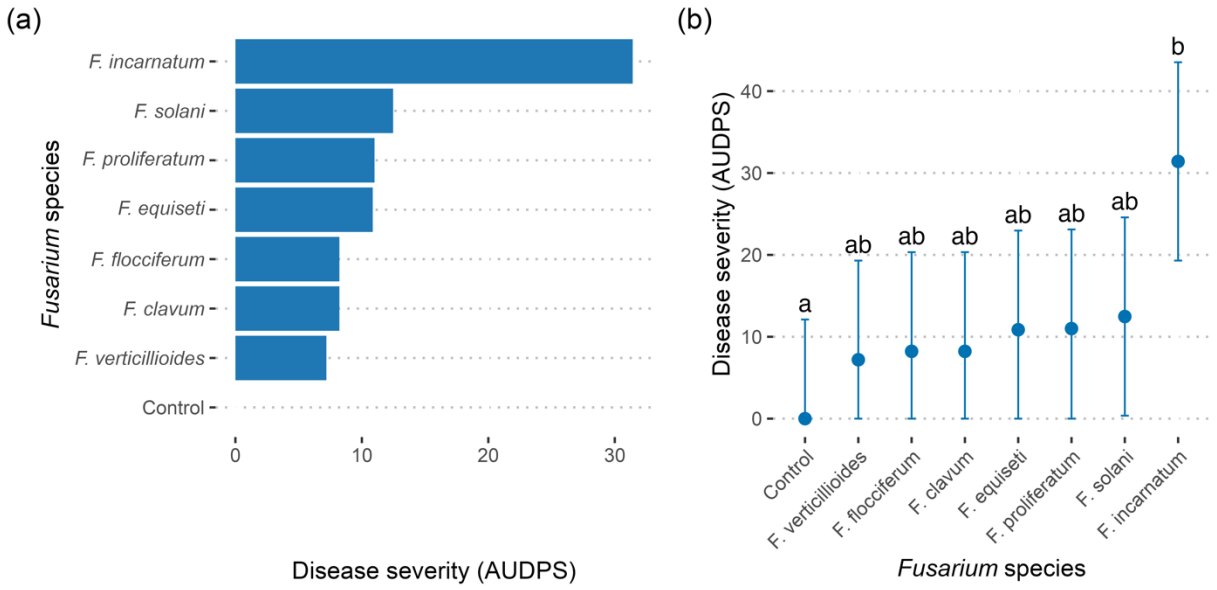

Fig 4. Comparison of the area under the disease progress stairs (AUDPS) based on severity ratings recorded on aerial parts of cotton seedlings across all four disease assessments: (A) AUDPS for each *Fusarium* species; (B) a linear mixed effect model fit showing estimated marginal means back-transformed from logit scale using the emmeans R package. Different letters (a, b) indicate significant differences among treatments at $P \leq 0.05$.

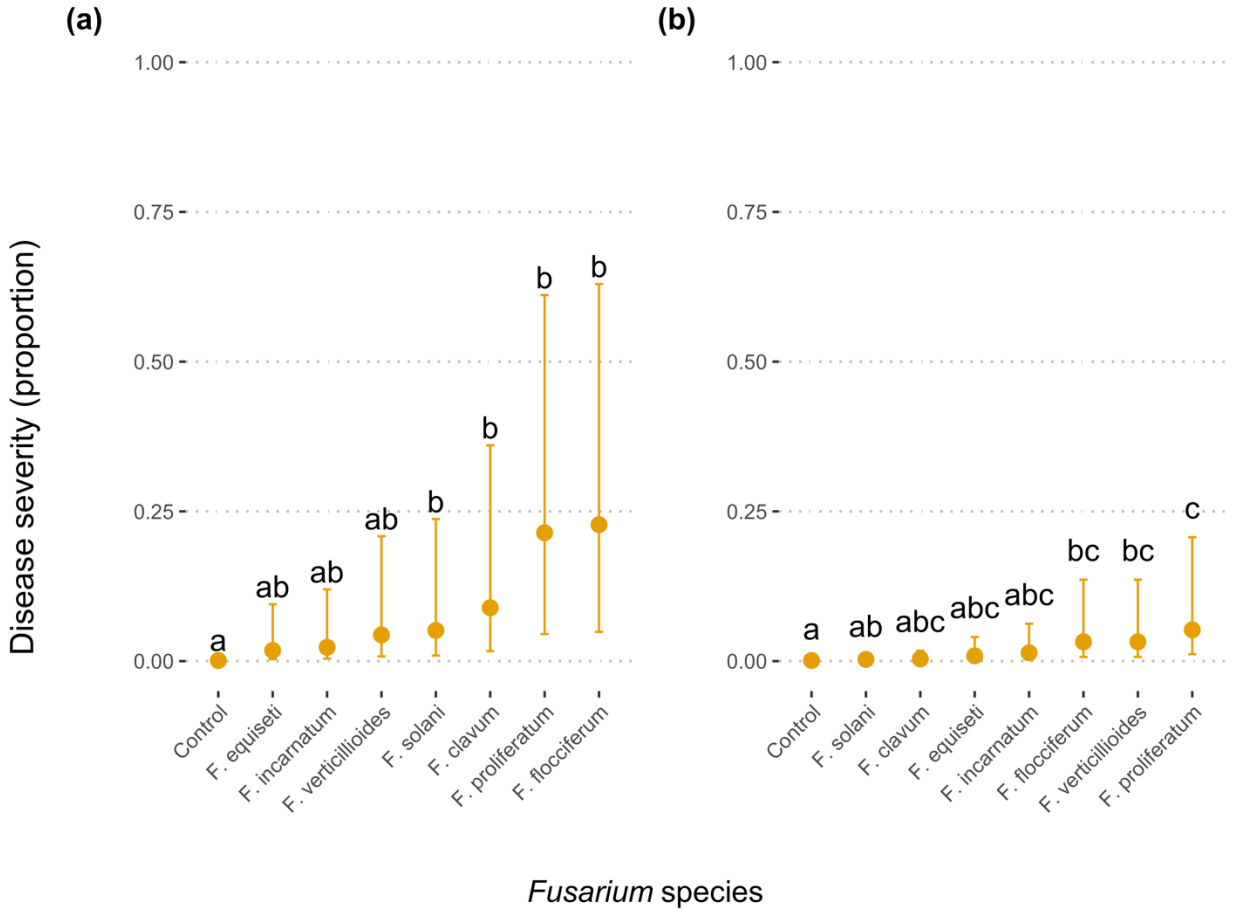

Fig. **5** A linear mixed effect model fit showing the disease severity of different *Fusarium* species on roots of (A) cotton and (B) corn. Different letters (a, b) indicate significant differences among treatments at $P \leq 0.05$. Probabilities and ± 1 standard error are shown as estimated marginal means back-transformed from logit scale using the emmeans R package.

564 **Table S1** Disease rating scales used for assessing disease severity of *Fusarium*

565 species in cotton and corn. Midpoints are expressed in percentage. Proportions were

566 obtained by dividing midpoint values by 100. For cotton, disease severity was recorded

567 on both aerial parts and roots, whereas for corn, it was recorded on roots only.

Crop	Plant part	Rating	Description	Range (%)	Midpoint	Proportion
Cotton	**Aerial parts**	0	Healthy	-	-	-
		1	Cotyledons wilted only	-	-	-
		2	≤50% true leaves wilted	-	-	-
		3	>50% but ≤90% true leaves wilted	-	-	-
		4	All leaves wilted or plant death	-	-	-
	Roots	0	no symptoms	-	-	-
		1	< 10% of roots discolored	1–9	5.00	0.05
		2	10–30% of roots discolored	10–30	20.00	0.20
		3	31–50% of roots discolored	31–50	40.50	0.41
		4	51–80% of roots discolored	51–80	65.50	0.66
		5	81–100% of roots discolored	81–100	90.50	0.91
Corn	**Roots**	1	No visible symptoms, 0% infection, and normal development	-	-	-
		2	Small speck of lesions near the crown and/or superficial root infection. Infection is <10%, and development is normal	1–9	5.00	0.05
		3	Superficial lesions visible near the crown or stem. Infection is 10–20%, and development is normal	10–20	15.00	0.15
		4	Deep lesion has advanced from crown and lower roots to the middle stem. Infection is 20–40%, and development is normal	21–40	30.50	0.31
		5	Deep lesion visible through approximately half the length of the stem and root. Infection is 40–60%, and plant development is affected	40–60	50.00	0.50
		6	Deep lesions on most parts of the stem and root, reduced root growth, significantly reduced plant development, and stunting are observed. Infection is 60–85%	60–85	72.50	0.73
		7	Severe deep lesions covering almost all parts of the stem and root, significantly reduced root growth and plant development, and extensive stunting are observed. Infection is 85–95%	85–95	90.00	0.90
		8	Seedling barely emerged and could not grow, scanty root but no emergence, or seed is dead (no emergence and no roots). Infection coverage of seedling is >95%	95–100	97.50	0.98

568

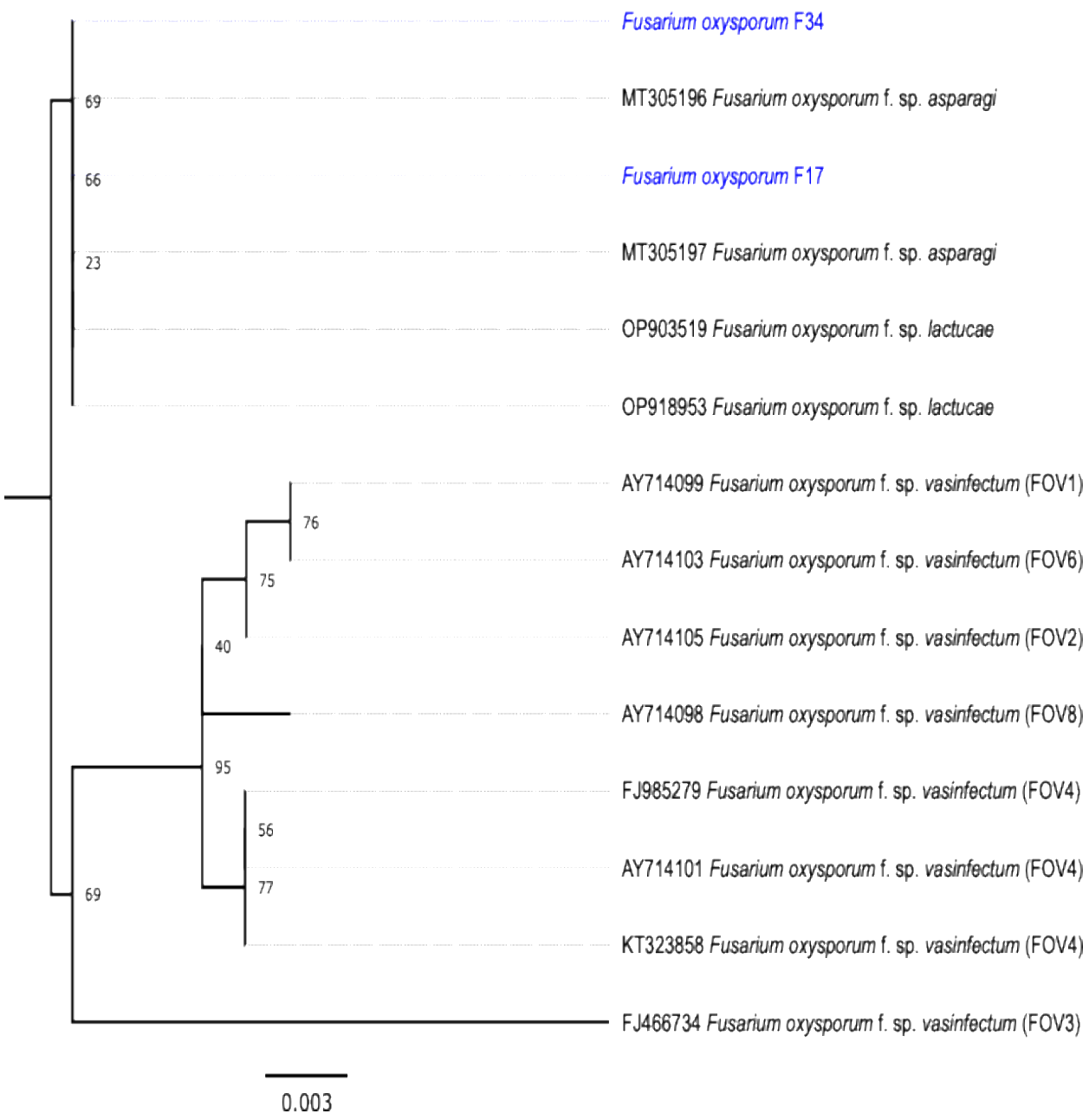

Fig. S1 Phylogenetic relationships of *Fusarium oxysporum* isolates collected in this study (highlighted in blue) compared with reference isolates of *F. oxysporum* f. sp. *vasinfectum* races 1, 2, 3, 4, 6, and 8 (FOV1 to FOV8) and other closely related species, based on sequences of the translation elongation factor 1-α (tef1-α) gene region.

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