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

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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. We investigated the diversity, distribution, and pathogenicity of *Fusarium* species in soil samples collected from 26 cotton-corn rotation fields across 10 counties. 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 (five species) 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 frequently isolated species (2%). Greenhouse pathogenicity assays showed *F. incarnatum* caused significantly higher disease severity relative to non-inoculated control on aerial parts of cotton (P=0.0137), while *Fusarium flocciferum*, *F. proliferatum,* and *F. clavum* caused significantly higher disease severity relative to non-inoculated control on roots (P=0.01, P=0.01, P=0.02, respectively). On corn, differences in disease severity were not statistically significant (P>0.05). *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 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). Additionally, 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). 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 crop due to water constraints and the high fertilizer costs associated with corn production (Colaizzi et al. 2009).

Cotton (*Gossypium* species) is the most important fiber crop for the textile industry and a significant oilseed crop globally. The United States is the world's fourth-largest cotton producing country (USDA Foreign Agricultural Service 2024), with cultivation spanning 17 southern states. Texas is the leading producer, accounting for approximately 40% of the U.S. cotton production in recent years (USDA Economic Research Service 2022). Although cotton was introduced to the Northern High Plains in 1968, acreage remained below 20,000 ha until 1998, but has expanded substantially

since 2004—including a 126% increase between 2013 and 2019 alone—raising concerns about diseases that may affect cotton production (Ledbetter 2020; Marek and Bordovsky 2006).

Key fungal genera implicated in cotton diseases worldwide and in the U.S. Cotton Belt include *Fusarium*, *Verticillium*, *Rhizoctonia*, and *Pythium*. Among these, *Fusarium*, *Rhizoctonia*, and *Pythium* species are primarily associated with the seedling disease complex (Howell 2002; Watkins 1981), while *Fusarium* species—alone or in combination with *Alternaria*, *Aspergillus* species, or bacterial pathogens such as *Pantoea agglomerans* and *Serratia marcescens*—are frequently linked to seed rot (Glover et al. 2020; Medrano et al. 2007; Minton and Garber 1983). Diseases caused by oomycetes and splash dispersed pathogens are not considered a major concern in the Northern High Plains due to the dry climate and severe water scarcity.

Fusarium wilt, caused by *Fusarium oxysporum* f.sp. *vasinfectum* (FOV), is of particular concern in the Northern High Plains because cooler temperatures during the early planting season favor its development (Zhang et al. 2020). Night temperatures in the Northern Panhandle are among the coolest in cotton growing regions of the U.S., with mean annual temperature of about 11°C (Kimmel Jr et al. 2016). 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

90 in the absence of root knot nematode *Meloidogyne incognita.* FOV4 was first detected
91 in the U.S. in 2001 in California (Kim et al. 2005) and more recently in 2017 in Texas
92 (Halpern et al. 2018) and 2019 in New Mexico (Zhu et al. 2019). This highly aggressive
93 race has not yet been reported in the Northern High Plains. However, given the
94 proximity to New Mexico and adjacent counties in Texas where FOV4 has been
95 detected (Cianchetta et al. 2015; Diaz et al. 2021), there is great concern about its
96 potential introduction into the Northern High Plains. Therefore, a comprehensive survey
97 is urgently needed to investigate the presence or absence of FOV4 in the region.
98 Several surveys have been conducted across the U.S. Cotton Belt for *Fusarium*
99 species (Cianchetta et al. 2015; Diaz et al. 2021; Mao and Hu 2019; Zhu et al. 2021).
100 However, these surveys did not include the Northern High Plains, leaving limited
101 information on the diversity, distribution and relative severity of *Fusarium* species
102 affecting cotton in this region. Moreover, these studies were primarily focused on
103 investigating the presence or absence of FOV4 rather than determining overall
104 *Fusarium* species diversity. Several *Fusarium* species are known to cause economically
105 important diseases in cotton, including wilt, damping-off, boll rot, and root rot (Davis et
106 al. 2006; Zaki et al. 2021). While *F. oxysporum* and *F. solani* are the most common
107 species complexes associated with cotton, the relative severity and impact of other
108 *Fusarium* species remain poorly understood (Mao and Hu 2019).
109 Crop rotation has been recognized as an effective practice to reduce nitrogen
110 fertilizer use and enhance both grain yield and sustainability in agricultural systems
111 (Shu et al. 2021). However, crop rotation can also inadvertently increase inoculum
112 levels, thereby elevating the risk of crop diseases (Li et al. 2021). Previous studies have

113 demonstrated that crop residue serves as a natural reservoir for *Fusarium* species and
114 may even facilitate genetic exchange (Ward et al. 2008). For instance, in wheat–corn
115 cropping rotations, continuous wheat cultivation following corn has been shown to
116 increase the incidence of *Fusarium* stalk rot and ear rot in maize, as well as *Fusarium*
117 head blight in wheat (Xi et al. 2021; Zhang et al. 2016). In the Northern High Plains,
118 where cotton and corn are commonly grown in rotation, investigating the pathogenicity
119 of *Fusarium* species on both crops is crucial. Such studies will provide valuable insights
120 into the pathogen's persistence, cross-infectivity, and the associated epidemiological
121 risks.

122 Greenhouse pathogenicity assays offer several advantages, including reduced
123 variability resulting from uneven inoculum distribution and external biotic or abiotic
124 factors. Most pathogenicity assays conducted in greenhouse conditions use root-dip
125 inoculation method, where two-week-old seedlings' roots are dipped in a spore
126 suspension for two minutes and then transplanted into healthy soil (Kim et al. 2005).
127 This method can predispose seedlings to stress during transplantation and does not
128 reflect host infection in the field (Schoeneweiss 1975). Conversely, pathogenicity
129 assays that involve directly planting seeds in *Fusarium*-infested soil or adding inoculum
130 after seedling emergence better reflect infection under field conditions (Diaz et al.
131 2021).

132 To address these key gaps, the present study was conducted to: (1) conduct a
133 comprehensive survey to determine the diversity and distribution of *Fusarium* species
134 associated with cotton-corn rotation fields in the recently expanded cotton-growing
135 areas of the Northern High Plains; (2) investigate the presence or absence of FOV4 in

136 the region; and (3) evaluate the pathogenicity and severity of *Fusarium* species on
137 cotton and corn under greenhouse conditions.

138 **Materials and Methods**

139 **Sampling sites and sampling procedure**

140 With the help of growers and county agents, a total of 26 cotton-corn rotation
141 fields across 10 different counties in the Northern High Plains of Texas were sampled
142 between late spring and late fall (May to October) of 2024. Specifically, six fields were
143 sampled in Sherman County; four fields each in Moore and Deaf Smith counties; three
144 fields in Hartley County; two fields each in Randall, Hansford, and Carson counties; and
145 one field each in Potter, Dallam, and Hutchinson counties. Fields selection was based
146 on growers or county agents' willingness to participate in the study.

147 In each field, a total of 25 soil cores, each 0.2 m deep, were collected at 5
148 different locations, with at least 60 m space between sampling points (5 soil cores per
149 sampling location within a field, 25 soil cores per field). The soil samples from each field
150 were pooled and placed into cloth bags, kept on ice in an insulated box to protect them
151 from high temperature and direct sunlight, and carried to the laboratory.

152 In the laboratory, the samples collected from each field were mixed thoroughly
153 and air dried for a week to inhibit microbial activity and facilitate grinding and sieving
154 (Sangalang et al. 1995). The air-dried soil sample for each field was poured into a
155 Nasco-Asplin soil grinder, pulverized gently to break the clods, and passed through a 2
156 mm sieve. To prevent cross-contamination between samples, the excess dirt from
157 grinder's parts were removed with a brush, washed with water, and then cleaned
158 thoroughly with 70% ethanol and paper towels.

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

160 **Isolation of single spore isolates from soil**

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

174 After incubating for two weeks on PDA, 200 µL of RO water was added to each
175 Petri dish, and conidia were dislodged by gently scrapping the mycelium with a pipette
176 tip. A total of 150 µL of this conidial suspension was then transferred onto 30% water
177 agar and incubated overnight. Single spore isolates were obtained by transferring single
178 germinated conidia, observed under Nikon SM225 microscope (Nikon corp., Tokyo,
179 Japan) at 180x magnification, onto fresh PDA plates.

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

181 Isolates were divided into morphotypes based on their gross colony morphology
182 (e.g., mycelial growth patterns), and their identities were obtained using the nuclear
183 ribosomal internal transcribed spacer region (ITS rDNA) and the translation elongation
184 factor 1-α (tef-1α) gene region (Table 1). DNA was extracted using Zymo Quick DNA
185 Fungal/Bacterial Microprep kit (Zymo Research Corp; Irvine, CA). Amplicons were
186 obtained using the following primer pairs: ITS1 (TCCGTAGGTGAACCTGCG) × ITS4
187 (TCCTCCGCTTATTGATATGC) for ITS rDNA (White et al. 1990); and EF-1 (5′-
188 ATGGGT AAGGARGACAAGAC-3′) × EF-2 (5′-GGARGTACCA GTSATCATG-3′) for tef-
189 1α gene region (O'Donnell et al. 1998). The PCR reaction mixture (25 µl reaction)
190 contained 12.5 µl of Phire Green Hot Start II Master Mix (Thermo scientific), 1.25 µl of
191 each primer (0. 5 µM), and 1.5 µl of genomic DNA. The PCR amplification program
192 conditions were as follows: initial denaturation at 98°C for 90 s followed by 35 cycles at
193 98°C for 5 s, annealing at 58°C for 15 s and extension at 72°C for 15 s, followed by final
194 extension for 4 minutes at 72°C.

195 The PCR products were detected using 1% agarose gel in 1x TAE buffer. The
196 PCR products were cleaned using DNA Clean & Concentrator kit (Zymo Research) and
197 sent to Eurofins Genomics (Eurofins Genomics LLC, Louisville, Kentucky) for
198 sequencing with both forward and reverse primers. ABI sequence chromatograms were
199 edited using SnapGene 8.0 (https://www.snapgene.com/) and exported as FASTA files
200 that were aligned using MUSCLE alignment (Edgar 2004) in Mega11
201 (https://www.megasoftware.net/). Consensus sequences were obtained using BioEdit
202 7.7.1 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html), which were then used to conduct

203 BLASTn queries of NCBI GenBank (https://www.ncbi.nlm.nih. gov/genbank/) to obtain
204 preliminary identifications.
205 Given the regional importance of *F. oxysporum*, two isolates identified as *F.*
206 *oxysporum* were additionally amplified using RNA polymerase II second largest subunit
207 (RPB2) (Liu et al. 1999) with the primer pair: 5f2 (GGGGWGAYCAGAAGAAGGC) × 7cr
208 (CCCATRGCTTGYTTRCCCAT), using the PCR conditions described above, to further
209 confirm its identity.
210 The two isolates identified as *F. oxysporum* in the present study were tested to
211 determine whether they are FOV4 (and to determine whether they are genotype N, T,
212 MT, and MiT) using specific primer pairs developed by Bell et al. (2019), following the
213 protocol described by Wagner et al. (2022). This PCR detection method has been
214 developed based on Tfo1 insertion event at the NAT gene locus and provides a rapid
215 alternative approach to vegetative compatibility analysis, which is labour-intensive and
216 time consuming (Wagner et al. 2022). Specific primer pairs included: FovT-F
217 (GGCCGATATTGTCGGTCGTA) x FovA-R (TCAACAGACCCTGCACTACG) for the
218 detection of FOV4; FovP-F (GGCCGATATTGTCGGTCGTA) x FovP-R
219 (CTCCAGTGCAGTGCTTGGTA) for the detection of FOV4 N genotype; FovP-F
220 (GGCCGATATTGTCGGTCGTA) x FovT-R (ATCTGTCTTTCGTCGGCAAT) for the
221 detection of FOV4 T or FOV4 MiT genotype; and FovP-F
222 (GGCCGATATTGTCGGTCGTA) x FovM-R (CCGCCATATCCACTGAACA) for the
223 detection of FOV4 MT genotype.
224 **Phylogenetic analyses**

225 Nucleotide sequences generated in this study, along with reference sequences
226 retrieved from the NCBI GenBank database, were aligned using MUSCLE alignment
227 (Edgar 2004) in Mega11 (https://www.megasoftware.net/). Alignments were then
228 trimmed avoiding missing data at either end of alignments. The aligned sequences were
229 analysed with maximum likelihood bootstrapping (MLBS) using IQ-TREE (http://
230 www.iqtree.org/) (Nguyen et al. 2015) to determine the best-fit model of molecular
231 evolution. These models were based on the Bayesian information criterion (BIC) scores
232 (Chernomor et al. 2016) using ModelFinder (Kalyaanamoorthy et al. 2017). Once the
233 optimal models were determined, clade support was assessed via 50,000 ML-BS
234 pseudoreplicates of the data using IQ-TREE.
235 To determine the phylogenetic placement of *Fusarium oxysporum* sequences
236 generated in this study relative to known FOV races (Cianchetta et al. 2015; Wang et
237 al. 2010) and closely related sequences, additional reference sequences representing
238 recognized FOV races and top BLASTn hits from the NCBI GenBank database were
239 retrieved and phylogenetic analysis was conducted as described above. DNA
240 sequences generated in the present study were deposited in NCBI (Table 1).
241 **Pathogenicity trials**
242 A single trial was conducted to test the pathogenicity and severity of seven
243 isolates, each representing a different *Fusarium* species, on corn and cotton. These
244 included *F. incarnatum, F. equiseti, F. solani, F. verticillioides, F. proliferatum, F.*
245 *clavum, and F. flocciferum.* Given its regional importance, *F. oxysporum* was not
246 included in the inoculated pathogenicity trials due to regional concerns in relation to

247 potentially working with FOV and the potential risk of its spread into these recently
248 expanded cotton-growing areas.

249 **Pathogenicity of *Fusarium* species on cotton**

250 **Biological material and experimental design**

251 The trial was conducted under greenhouse conditions at Texas A&M AgriLife
252 Research, Bushland, Texas at ambient temperatures (25 ± 5°C). A commonly cultivated
253 variety in the Texas High Plains, FiberMax 868AXTP, with fair resistance to Fusarium
254 wilt was used in this study (BASF 2024). Disease-free seeds of this variety were
255 sourced from a previous trial: seeds were de-linted but received no chemical treatment
256 prior to planting. Briefly, 10-cm pots were filled with a modified media (1 bag of Berger's
257 OM1 potting mix, 0.085 m^3 of organic growing mix, 2.2 kg of sand, and 3 kg of soil). A
258 total of 2-3 disease-free seeds were sown in each pot. Upon germination, seedlings
259 were thinned to one per pot. There were six replicate pots for each *Fusarium* species
260 and a non-inoculated control (total 48 plants), arranged in a completely randomized
261 block design. Throughout the trial period, seedlings were fertilized with water-soluble
262 fertilizer 'Miracle-Gro' as needed.

263 **Inoculum preparation**

264 The sorghum grain inoculation method was used to prepare the inoculum (Yadav
265 et al. 2017). Disease-free sorghum grains were sourced from a previous year's trial.
266 Briefly, sorghum grains were soaked overnight, and 50 g of soaked grains were placed
267 into each 250 mL beaker and sealed with aluminium foil. The beakers were autoclaved
268 at 121°C for 30 minutes on two consecutive days. Once cooled, the sorghum grains in
269 each beaker were inoculated with five agar plugs (5 mm), which were cut from a 7-day-

270 old colony of specific *Fusarium* isolate grown on PDA. The inoculated beakers were
271 gently shaken to uniformly distribute the inoculum and then placed inside zip-lock plastic
272 bags to avoid contamination. The inoculated beakers were incubated for 7 days at room
273 temperature (23 ± 2°C) with a 12-hour photoperiod and shaken every 2–3 days to
274 ensure even distribution of the inoculum.

275 **Inoculation method**

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

283 **Disease assessment**

284 Cotton seedlings were observed daily for the appearance of disease symptoms.
285 Disease severity on aerial plant parts was recorded at 7, 14, 21, and 28 days post-
286 inoculation using a 0–4 rating scale, where: 0 = healthy; 1 = cotyledons wilted only; 2 =
287 ≤50% true leaves wilted; 3= >50% but ≤90% true leaves wilted; and 4 = all leaves wilted
288 or plant death (Table S1).

289 All cotton seedlings were harvested after one month of inoculation. Roots were
290 washed free of soil and disease severity was recorded using 0–5 rating scale, where 0 =
291 no symptoms; 1 = <10% of roots discolored; 2 = 10–30% of roots discolored; 3= 31–

292 50% of roots discolored; 4 = 51–80% of roots discolored; and 5 = 81–100% of roots
293 discolored (Table S1).
294 At the end of the trial, the roots of all cotton seedlings were plated onto acidified
295 PDA (1000 mL RO water, 39 g potato dextrose agar, 1 g streptomycin) to confirm
296 pathogenicity.

297 **Pathogenicity of *Fusarium* species on corn**

298 **Biological material, experimental design, inoculum preparation and inoculation**
299 **method**

300 A commonly cultivated drought tolerant, untreated corn hybrid, Blue River
301 Organic 82-14P, was used in the pathogenicity trial.
302 The experimental design, inoculum preparation and application method, as well
303 as cultural practices, were the same as those used in the cotton pathogenicity trial. That
304 is, a single batch of inoculum was used to inoculate both cotton and corn seedlings at
305 the same time and similar cultural practices were followed to ensure consistency.
306 Seedlings were inoculated at the three-leaf stage.

307 **Disease assessment**

308 Corn seedlings were harvested after one month of inoculation. Roots were
309 washed free of soil and crown and root lesions were rated using a 1–8 rating scale
310 (Parikh et al. 2018), where: 1 = no visible symptoms, 0% infection, and normal
311 development; and 8 = seedling barely emerged and could not grow, scanty root but no
312 emergence, or seed is dead (no emergence and no roots); infection coverage of
313 seedling is >95% (Table S1).

314 At the end of the trial, the roots of all corn seedlings were plated onto acidified
315 PDA (1000 mL RO water, 39 g potato dextrose agar, 1 g streptomycin) to confirm
316 pathogenicity.

317 **Data Analysis**

318 In the case of disease severity recorded on aerial parts of cotton, the area under
319 the disease progress stairs (AUDPS) was calculated using the 'audps()' function from
320 'agricolae' R package (Mendiburu 2023), based on severity ratings recorded across all
321 four disease assessments. The AUDPS was selected over the traditional area under the
322 disease progress curve (AUDPC) because the latter underestimates the effect of
323 disease severity recorded during first and last disease assessments (Simko and Piepho
324 2012).

325 Linear mixed models effect models (LMMs) were then fitted using the 'lmer()'
326 function from the 'lmerTest' R package (Kuznetsova et al. 2022) to investigate
327 differences in disease severity among *Fusarium* species. The AUDPS was included as
328 the dependent variable, the predictor *Fusarium* species were included as a fixed effect,
329 and different blocks were included as a random effect to account for variability across
330 blocks. Estimated marginal means for each *Fusarium* species were calculated using the
331 'emmeans()' function from 'emmeans' R package (Lenth 2021). The estimated marginal
332 means were compared using Tukey-adjusted pairwise comparisons, and groupings
333 were assigned using the 'cld()' function from the 'multcomp' R package (Hothorn 2020).
334 These groupings indicated significant differences at $\alpha = 0.05$. Models diagnostics was
335 performed using the 'DHARMa' R package, which uses a simulation-based approach

336 via the function ‘simulateResiduals’ to create readily interpretable scaled residuals for
337 models (Hartig 2020).
338 In the case of disease severity recorded on roots of cotton and corn, differences
339 in disease severity were tested using a nonparametric Kruskal-Wallis test using
340 ‘ggbetweenstats()’ function from the ‘ggstatplot’ package (Patil 2021). If significant
341 differences were found (α = 0.05), pairwise differences were performed using a post hoc
342 Dunn test.

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). The number of isolates recovered for
348 each species and their counties of origin are given in Table 1. Colony morphology (e.g.,
349 mycelial growth patterns) on PDA varied among isolates belonging to the same species,
350 particularly for *F. solani.* No PCR amplifications were achieved for two *F. oxysporum*
351 isolates identified in this study using FOV4 specific primers. Phylogenetic analysis of
352 tef-1α gene region placed these two *F. oxysporum* isolates within a clade containing
353 reference sequences of *F. oxysporum* f. sp. *asparagi* and *F. oxysporum* f. sp. *lactucae*
354 (Figure S1). *Fusarium solani* was the most frequently isolated species (39%), followed
355 by *F. equiseti* (22%), *F. proliferatum* and *F. clavum* (7% each), *F. incarnatum*, *F.*
356 *verticillioides*, *F. oxysporum*, and *Fusarium* sp. (5% each), and *F. flocciferum* (2%)
357 (Table 1).

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

366 **Pathogenicity of *Fusarium* species on cotton**

367 All *Fusarium* isolates tested were pathogenic on cotton; however, disease
368 severity was largely restricted to root discoloration (Figure 2). No disease was recorded
369 on non-inoculated control plants. *Fusarium* isolates were re-isolated from all inoculated
370 roots, both symptomatic and asymptomatic, plated on PDA (i.e., 100% re-isolation rate).
371 For the aerial parts, the highest disease severity (expressed as AUDPS) was
372 caused by *F. incarnatum* (31.4), followed by *F. solani* (12.5), while the lowest disease
373 severity was associated with *F. verticillioides* (7.1) (Figure 3A). Only *F. incarnatum*
374 caused significantly higher disease severity relative to non-inoculated control (Figure
375 3B, P=0.0137, EMMeans pairwise comparisons). Disease severity associated other
376 *Fusarium* species did not differ significantly from the non-inoculated control plants or
377 from each other (Figure 3B, P>0.05, EMMeans pairwise comparisons). DHARMa
378 diagnostics showed that the data met the model assumptions.
379 For roots, the Kruskal-Wallis test indicated that there was a significant difference
380 in disease severity among groups (Figure 4A, χ^2 = 17.58, df = 5, P = 0.01). The highest

381 disease severity, expressed as median rank, was caused by *F. proliferatum* and *F.*
382 *clavum* (2 each) (Figure 4A). *Fusarium flocciferum*, *F. proliferatum,* and *F. clavum*
383 caused significantly higher disease severity relative to non-inoculated control (Figure
384 4A, P=0.01, P=0.01, P=0.02, respectively). However, no significant differences in
385 disease severity were recorded among different *Fusarium* species (Figure 4A, P>0.05).

386 **Pathogenicity of *Fusarium* species on corn**

387 All *Fusarium* isolates tested were pathogenic on corn, causing stalk rot, root
388 necrosis and brown to black discolorations; however, disease severity did not exceed
389 20–40%. The highest disease severity, expressed as median rank, was caused by *F.*
390 *proliferatum, F.* flocciferum, *F. incarnatum* and *F. verticillioides* (2 each) (Figure 4B). No
391 disease was recorded on non-inoculated control plants. Like cotton, *Fusarium* species
392 were re-isolated from all inoculated corn plants, regardless of whether they exhibited
393 symptoms. Differences in disease severity were not statistically significant among
394 *Fusarium* species or relative to non-inoculated control plants (Figure 4B, P>0.05).

395 **Discussion**

396 To the best of our knowledge, this study is the first to conduct a comprehensive
397 survey of the recently expanded cotton-growing areas in the Northern High Plains of
398 Texas and provided valuable insights into the diversity, distribution, and pathogenicity
399 of *Fusarium* species associated with cotton-corn rotation fields. A total of nine *Fusarium*
400 species were identified, with *F. solani* being the most frequently isolated and *F.*
401 *flocciferum* the least. Sherman County harboured the highest number of *Fusarium*
402 species. All tested species were pathogenic on cotton and corn during the pathogenicity
403 assays conducted under greenhouse conditions.

404 The high frequency of *F. solani* and *F. equiseti* isolation in the present study
405 aligns with prior findings from Arizona and Alabama, where they were the most
406 frequently isolated species from cotton roots and rhizosphere soil (Mao and Hu 2019;
407 Palmateer et al. 2002). Likewise, *F. solani* was reported as the most predominant
408 species, isolated from 97% of sorghum and corn fields across central and southeastern
409 United States (Leslie et al. 1990). Likewise, the least frequent isolation of *F. flocciferum*
410 in the present study is consistent with the findings from previous studies conducted in
411 France (Nguyen et al. 2024) and Belgium (Scauflaire et al. 2011), where it was among
412 the least frequently isolated species in corn agroecosystems. To date, there are no
413 reports of *F. flocciferum* as a widely distributed pathogen in the U.S. Cotton Belt. These
414 findings suggest that *F. solani* and *F. equiseti* are habitat generalists, being widely
415 distributed across the U.S. Cotton Belt, whereas *F. flocciferum* is mostly a habitat
416 specialist with a narrow distribution range.
417 The observation that colony morphology (e.g., mycelial growth patterns) on PDA
418 varied among isolates of the same species, particularly *F. solani,* is consist with finding
419 from a previous study (Mao and Hu 2019) and suggests that morphological
420 characteristics alone are insufficient for species identification, emphasizing the need for
421 sequence-based identification.
422 The variation in *Fusarium* species diversity observed across counties in this
423 study is likely caused by a combination of factors, including environmental conditions,
424 anthropogenic disturbance, variation in the number of fields sampled across counties,
425 soil types, cultural practices (e.g., crop rotation and tillage), and predisposing factors
426 such as the presence of nematodes or flooding events (Mai and Abawi 1987;

427 Steinkellner and Langer 2004). Differences in cotton acreage among counties may have
428 also contributed to variation in species diversity across counties. Specifically, the
429 highest species diversity in Sherman, Moore, and Carson counties can partly be
430 attributed to the substantial increase in cotton acreage between 2013 and 2018, during
431 which production grew by 301% to over 250,000 hectares (Ledbetter 2020; Marek and
432 Bordovsky 2006).

433 The significantly higher disease severity observed on cotton inoculated with *F.*
434 *flocciferum*, *F. proliferatum*, and *F. clavum*, and *F. solani* (relative to non-inoculated
435 control plants), suggests that these species may pose a potential threat to cotton and
436 corn production in the Northern High Plains. Among these, *F. proliferatum* and *F. solani*
437 have been previously identified as the most virulent species on cotton (Abdel-Latif et al.
438 2007; Palmateer et al. 2002). *Fusarium proliferatum* has also been reported as a
439 pathogen of cotton in New Mexico (Zhu et al. 2019). Disease severity on corn did not
440 differ significantly between inoculated and control plants or among *Fusarium* species;
441 however, *F. proliferatum* and *F. verticillioides* have been described as the most virulent
442 species on corn in previous studies (Okello et al. 2019; Wang et al. 2021). The
443 pathogenicity of *Fusarium* species on both cotton and corn suggests that they can
444 overwinter on the stubble of either crop and serve as a primary source of inoculum in
445 the next spring.

446 The observation of overall low disease severity on both cotton and corn may
447 partly be attributed to the application of inoculum after seedling emergence for both
448 cotton and corn, rather than the use of a root-dip inoculation method. Although the root-
449 dip method can result in increased disease severity, it does not closely mimic natural

450 infection under field conditions and may introduce stress during transplantation,
451 potentially predisposing seedlings to infection (Diaz et al. 2021; Schoeneweiss 1975).
452 The 100% re-isolation rate of *Fusarium* species from both symptomatic and
453 asymptomatic roots following pathogenicity trials in the present study supports the
454 previous findings that systemic colonisation can occur in the absence of visible disease
455 symptoms (Leslie et al. 1990). This implies that the pathogenicity of *Fusarium* species in
456 the field may need to be interpreted with caution, as *Fusarium* species may be living in
457 plants as endophytes or secondary invaders (Leslie et al. 1990). In the case of plant
458 tissues, this further underscores the importance of sampling both symptomatic and
459 asymptomatic plant tissues for accurately determining the diversity and distribution of
460 *Fusarium* species (Leslie et al. 1990). The present study likely provided an accurate
461 estimate of *Fusarium* species diversity and distribution because isolates were recovered
462 from soil samples, which minimized the bias associated with symptom-based tissue
463 sampling and isolation. Furthermore, a previous study did not find any significant
464 differences in the number of *Fusarium* species isolated from cotton roots and
465 rhizosphere soil (Mao and Hu 2019), suggesting that soil-based isolation approaches
466 can reliably capture species diversity.
467 This study provided baseline *Fusarium* species in the Northern High Plains of
468 Texas. The detection of both common (e.g., *F. solani* and *F. equiseti*) and less
469 frequently reported species (e.g., *F. clavum* and *F. flocciferum*) highlights the diversity
470 of *Fusarium* species in this region. The non-detection of the highly aggressive FOV4,
471 using FOV4 specific primers, in present study suggests that the pathogen may not yet
472 have been introduced into the Northern High Plains. Additionally, the phylogenetic

clustering of the two *F. oxysporum* isolates recovered in the present study with reference sequences of *F. oxysporum* f. sp. *asparagi* and *F. oxysporum* f. sp. *lactucae* suggest that they unlikely belong to the forma specialis `vasinfectum`. Therefore, there is a probability that *F. oxysporum* isolates recovered in this study may have not been pathogenic on cotton. 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 (Fulcher et al. 2021), 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.

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496 **Author-Recommended Internet Resources**

497 https://github.com/IhsanKhaliq/HighPlainsFusa

498 **Literature Cited**

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692 **Table 1** Isolate identity, collection date, county of origin, host crop history, and GenBank accession numbers for *Fusarium*

693 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		

Isolate	Organism	County	Previous crop	Current crop	Sampling date	GenBank Accession number		
						tef-1α	ITS	RPB2
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	PV699150
F34	*F. oxysporum*	Carson	Corn	Cotton	2024/10/24	PV717849	PV731268	PV699151
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		

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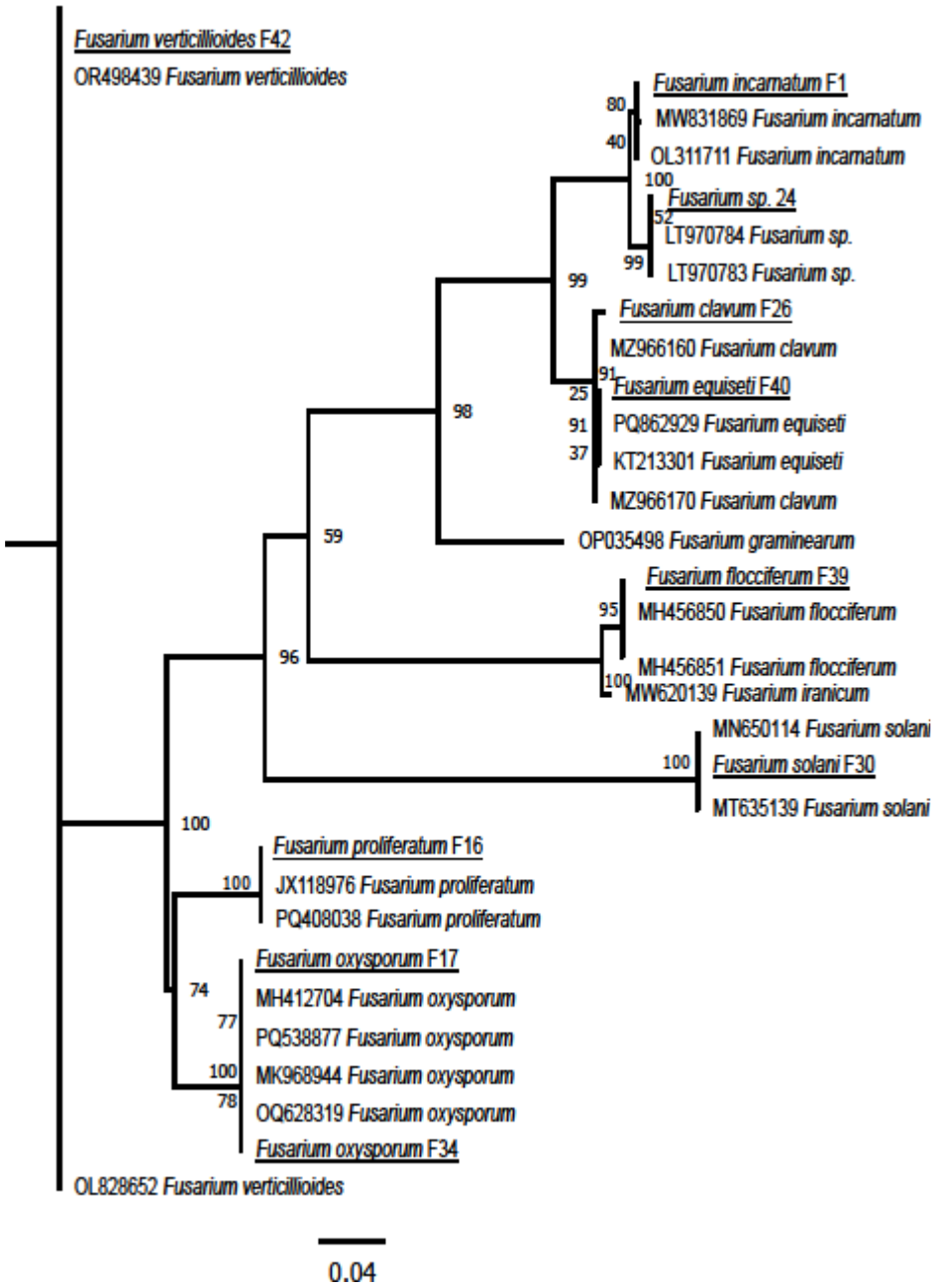

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698 **Figure** 1 Phylogenetic comparison of *Fusarium* species isolated from cotton-corn

699 rotation fields in the Texas High Plains (underlined) in relation to closely related

700 reference species based on the translation elongation factor 1-α (tef-1α) gene region.

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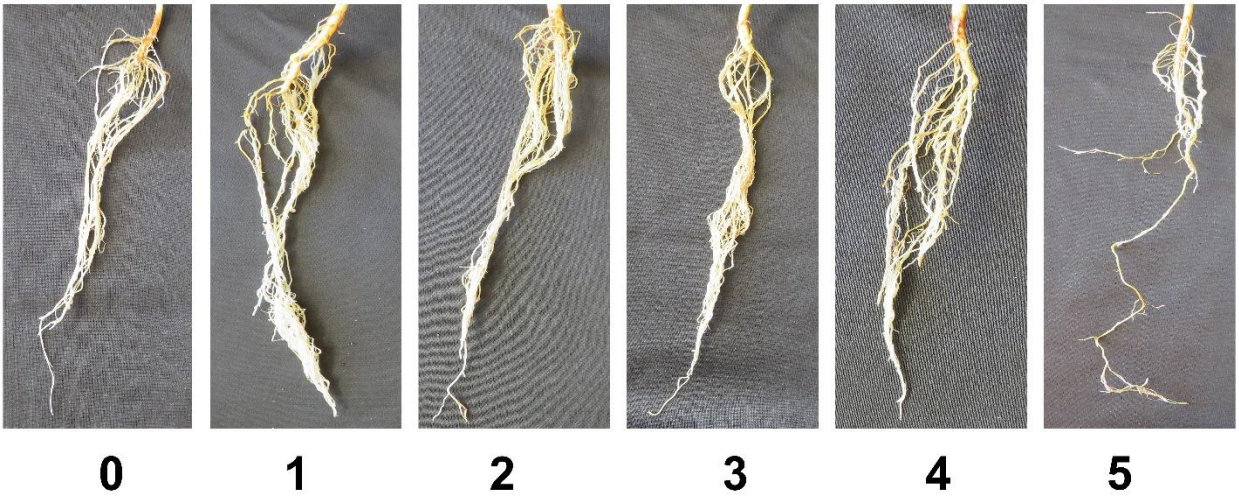

Figure 2 Damage on roots of cotton seedlings inoculated with *Fusarium* species. Whole root systems were rated visually using a 0–5 rating scale. Left to right: 0 - no symptoms; 1 - <10% of roots discolored; 2 - 10–30% of roots discolored; 3 - 31–50% of roots discolored; 4 - 51–80% of roots discolored;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

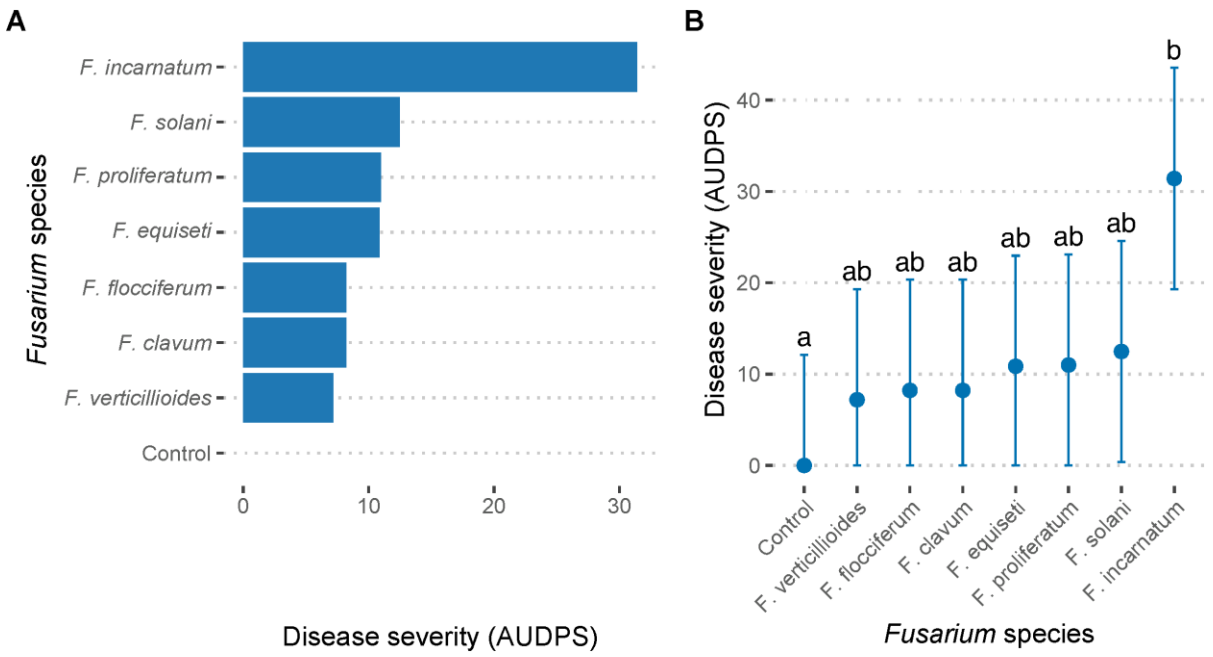

Figure 3. Comparison of the area under the disease progress stairs (AUDPS) based on severity ratings recorded on aerial parts of cotton seedlings across all four disease assessments: (A) AUDPS for each *Fusarium* species; (B) a linear mixed effect model fit showing estimated marginal means using the emmeans R package. Different letters (a, b) indicate significant differences among treatments at $P \leq 0.05$.

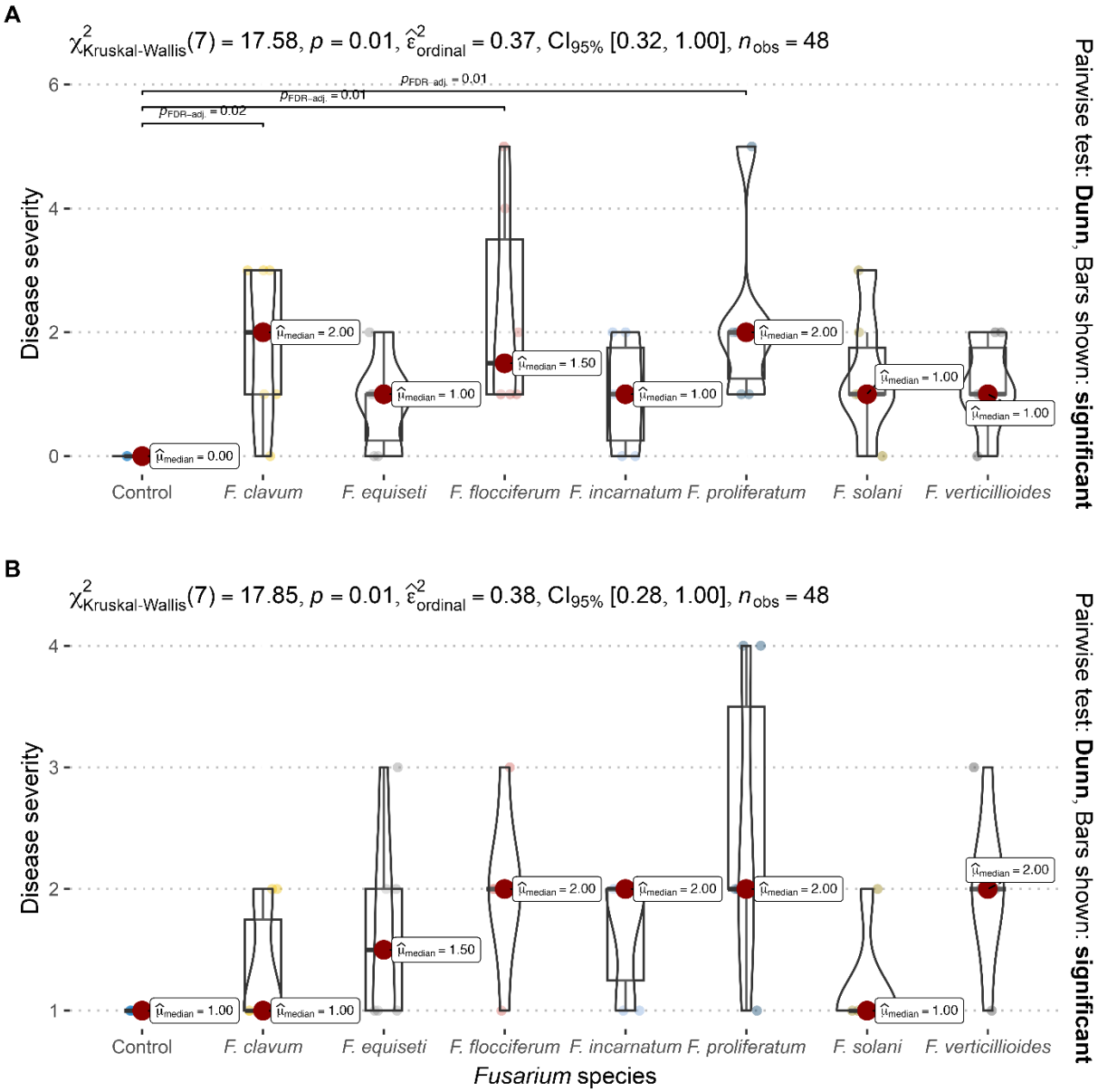

Figure 4 Comparison of disease severity of different *Fusarium* species on roots of (A) cotton and (B) corn. Differences in disease severity were compared using a non-parametric Kruskal–Wallis test.

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

741 species in cotton and corn. For cotton, disease severity was recorded on both aerial

742 parts and roots, whereas for corn, it was recorded on roots only.

Crop	Plant part	Rating	Description	Range (%)
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
		2	10–30% of roots discolored	10–30
		3	31–50% of roots discolored	31–50
		4	51–80% of roots discolored	51–80
		5	81–100% of roots discolored	81–100
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
		3	Superficial lesions visible near the crown or stem. Infection is 10–20%, and development is normal	10–20
		4	Deep lesion has advanced from crown and lower roots to the middle stem. Infection is 20–40%, and development is normal	21–40
		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

Crop	Plant part	Rating	Description	Range (%)
		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
		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
		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

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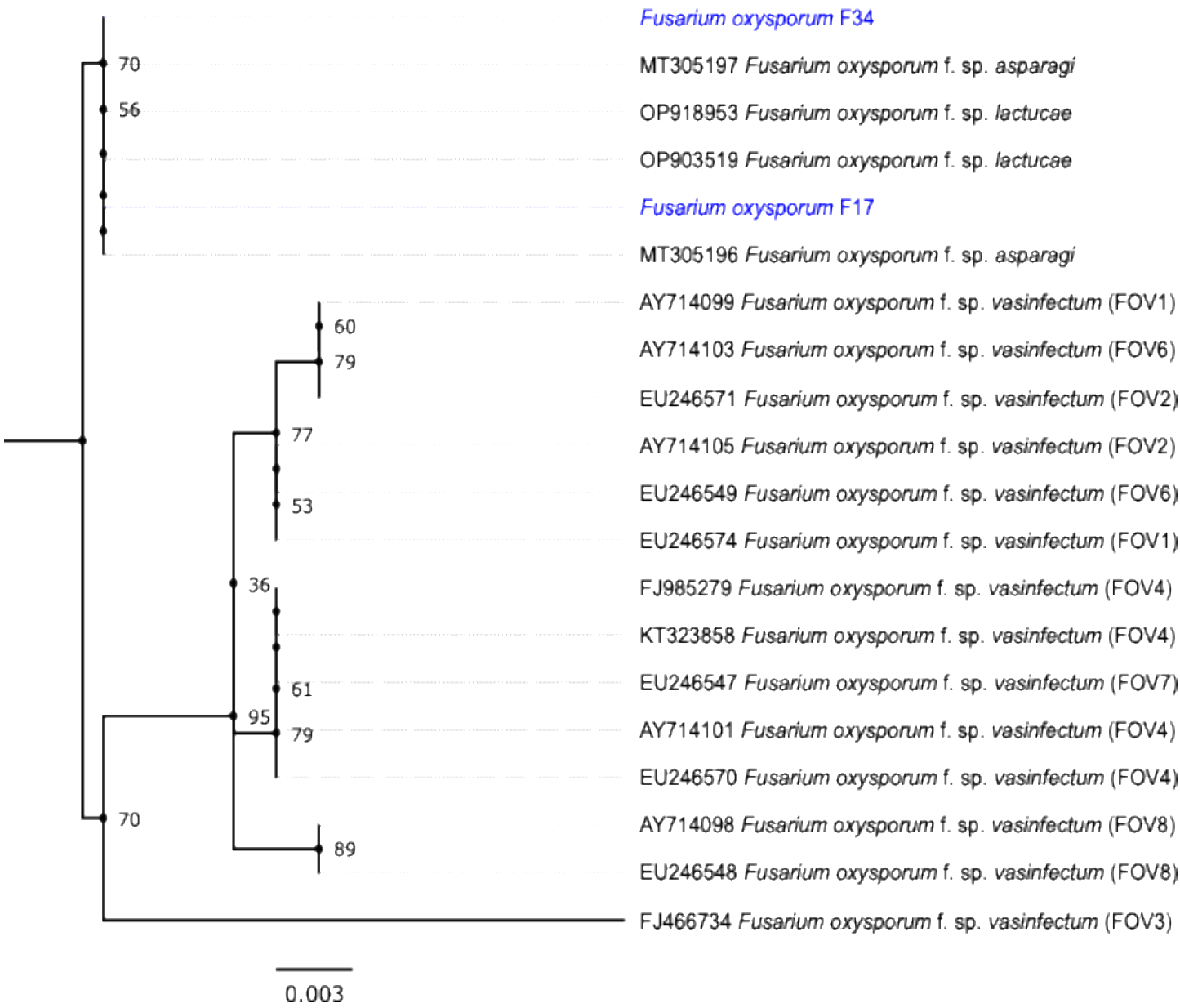

Fig. S1 *Phylogenetic* relationships of *Fusarium oxysporum* isolates collected in this study (highlighted in blue) compared with reference isolates of *F. oxysporum* f. sp. *vasinfectum* races 1, 2, 3, 4, 6, and 8 (FOV1 to FOV8) and other closely related species, based on sequences of the translation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