Plant Pathology (2019) **68**, 1654–1662 Doi: 10.1111/ppa.13082

Phylogenetic relationships and virulence assays of *Fusarium secorum* from sugar beet suggest a new look at species designations

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Fusarium spp. are responsible for significant yield losses in sugar beet (*Beta vulgaris*) with *Fusarium oxysporum* f. sp. *betae* most often reported as the primary causal agent. Recently, a new species, *F. secorum*, was reported to cause disease in sugar beet but little is known on the range of virulence within *F. secorum* or how this compares to the virulence and phylogenetic relationships previously reported for *Fusarium* pathogens of sugar beet. To initiate this study, partial translation elongation factor 1-α (*TEF1*) sequences from seven isolates of *F. secorum* were obtained and the data were added to a previously published phylogenetic tree that includes *F. oxysporum* f. sp. *betae*. Unexpectedly, the *F. secorum* strains nested into a distinct group that included isolates previously reported as *F. oxysporum* f. sp. *betae*. These results prompted an expanded phylogenetic analysis of *TEF1* sequences from genomes of publicly available *Fusarium* spp., resulting in the additional discovery that some isolates previously reported as *F. oxysporum* f. sp. *betae* are *F. commune*, a species that is not known to be a sugar beet pathogen. Inoculation of sugar beet with differing genetic backgrounds demonstrated that all *Fusarium* strains have a significant range in virulence depending on cultivar. Taken together, the data suggest that *F. secorum* is more widespread than previously thought. Consequently, future screening for disease resistance should rely on isolates representing the full diversity of the *Fusarium* population that impacts sugar beet.

Keywords: Fusarium commune, *Fusarium oxysporum* f. sp. *betae*, *Fusarium secorum*, fusarium yellowing decline, fusarium yellows

Introduction

Sugar beet (*Beta vulgaris*) is an important source of sucrose for the natural sweetener industry. Since 1990, sugar beet has accounted for a growing percentage of the United States sucrose production, now contributing greater than 50% of sucrose supplies (McConnell, 2015). *Fusarium* spp. can lead to significant economic losses for sugar beet growers by causing reductions in yield from several associated diseases (Campbell *et al.*, 2011) including fusarium yellows, primarily caused by *F. oxysporum* f. sp. *betae* (Stewart, 1931; Ruppel, 1991; Hanson & Hill, 2004), fusarium tip root caused by *F. oxysporum* f. sp. *radicis-betae* (Harveson & Rush, 1998), and fusarium stalk blight caused by *F. solani* and *F. oxysporum* (Hanson & Hill, 2004; Panella & Lewellen, 2005). Fusarium yellows was first described by Stewart (1931) from sugar beet roots with symptoms from the Arkansas Valley in south-eastern Colorado. Since that time, this disease has been reported in sugar beet production regions throughout North America (Ruppel, 1991; Hanson & Hill, 2004; Windels *et al.*, 2005; Hanson & Jacobsen, 2006) and Europe (Nitschke *et al.*, 2009).

In 2008, a new disease was reported from Minnesota that caused fusarium yellows-like symptoms. However, symptoms differed from the traditional fusarium yellows by also causing a half-leaf discoloration and yellowing that occurred from older to younger leaves (Rivera *et al.*, 2008; Burlakoti *et al.*, 2012). Subsequent studies confirmed that the causal agent of this disease was different from any previously described *Fusarium* species; therefore, it was named *F. secorum* and the disease was called fusarium yellowing decline (Secor *et al.*, 2014).

Fusarium oxysporum from sugar beet can be highly variable in growth, pigmentation, conidia production and virulence (Ruppel, 1991; Harveson & Rush, 1997;

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Published online 4 September 2019

Plant Pathology

Hanson & Hill, 2004; Hanson & Jacobsen, 2006; Hanson *et al*., 2009; Hill *et al*., 2011; Webb *et al*., 2012, 2017). Previous studies used genetic markers to characterize *F. oxysporum* isolates collected from sugar beet and discovered that genetic relatedness of the isolates did not necessarily correlate with pathogenicity, that virulence to sugar beet probably evolved from multiple sources, and genetic variation within *F. oxysporum* f. sp. *betae* is organized loosely into three groups designated at the time as A, B and C (Hill *et al*., 2011; Webb *et al*., 2012; Covey *et al*., 2014). In their studies, Secor *et al*. (2014) showed that the new sugar beet pathogen *F. secorum* was nested within the *F. fujikuroi* species complex (FFSC).

Sugar beet has been shown to have a varying response when inoculated with different isolates of *F. oxysporum* f. sp. *betae* and this has been attributed to genotype by isolate interactions (Ruppel, 1991; Hanson *et al*., 2009, 2018). While Burlakoti *et al*. (2012) screened multiple isolates of *F. secorum* for pathogenicity to a single line of sugar beet, little is known on the range of virulence within *F. secorum* across several sugar beet varieties or how virulence relates to the diversity of fusaria previously analysed from this host. The main objective of this research was to assess virulence of *F. secorum* to multiple sugar beet lines and better understand the phylogenetic relatedness of *F. secorum* isolates to the sugar beet *Fusarium* population previously reported.

Materials and methods

Fusarium isolates and maintenance of cultures

Fusarium isolates used for these studies were obtained from the long-term culture collections located at either the United States Department of Agriculture – Agricultural Research Service (USDA-ARS) Soil Management and Sugar Beet Research Unit in Fort Collins, CO, USA or at the Department of Plant Pathology, North Dakota State University (Table 1). Each isolate was originally recovered from a single conidium or via hyphal-tip transfer from cultures obtained from sugar beet with symptoms and stored on filter paper at −20 °C using modified protocols, as described by others (Leslie & Summerell, 2006). Working cultures of all isolates were maintained on potato dextrose agar plates (PDA; Becton, Dickinson & Co.) at room temperature until used, and transferred using established protocols (Leslie & Summerell, 2006). To validate identification of each isolate as either *F. secorum* or *F. oxysporum* f. sp. *betae*, each isolate was grown on half-strength PDA and carnation leaf agar at 25 °C under white fluorescent lights for 3–4 weeks.

DNA extractions and PCR amplification of translation elongation factor 1-α (*TEF1*)

Fusarium isolates were grown individually in 50 mL potato dextrose broth (PDB; Becton, Dickinson & Co.) by inoculating with a 7 mm diameter plug of mycelium taken from a growing culture. Liquid cultures were grown in the dark for 5–7 days at 25 °C on a rotary shaker at 100 rpm. Mycelial masses were collected by pouring the filtrate through a double layer of sterile cheesecloth, rinsed with deionized water, and then lyophilized at –50 °C for 48 h. Lyophilized tissue was ground into a fine powder using a spatula, and DNA extracted using an Easy-DNA extraction kit (Invitrogen) using the manufacturer's protocol.

TEF1 primers (EF1/EF2) were used for PCR amplification (O'Donnell *et al*., 1998) using a *Taq* DNA polymerase kit following the manufacturer's instructions (Thermo Scientific) and the following PCR conditions: one cycle of 94 °C for 5 min; followed by 33 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min; a final extension cycle of 72 °C for 5 min, followed by holding at 4 °C. Reactions were performed in a MasterCycler gradient thermocycler (Eppendorf). Amplicons were visualized on a 1.5% agarose gel and purified using the GenCatch PCR extraction kit (Epoch). Products were sequenced by Eurofins, MWG/Operon (Huntsville, AL). *TEF1* sequences were manually edited and consensus sequences generated using a pairwise sequence alignment in GENIOUS v. 6.1.8. Novel gene sequences from *F. secorum* isolates included in this study were submitted to GenBank under accession numbers MH926020–MH926026.

Table 1 List of *Fusarium* isolates used for assays of virulence on sugar beet.

Isolate name	Species[a]	Species ID after studies	Donor[b]	Year collected	Location collected
670-10	*F. secorum*	*F. secorum*	G. Secor	2005	Sabin, MN
742-28	*F. secorum*	*F. secorum*	G. Secor	2006	Sabin, MN
784-24-2C	*F. secorum*	*F. secorum*	G. Secor	2007	Sabin, MN
845-1-18	*F. secorum*	*F. secorum*	G. Secor	2010	Foxhome, MN
938-4	*F. secorum*	*F. secorum*	G. Secor	2010	Moorhead, MN
938-6	*F. secorum*	*F. secorum*	G. Secor	2007	Sabin, MN
1090-4-2	*F. secorum*	*F. secorum*	G. Secor	2012	Fargo, ND
F19	*F. oxysporum* f. sp. *betae*	*F. commune*	L. Hanson	2001	Salem, OR
Fob220a	*F. oxysporum* f. sp. *betae*	*F. secorum*	H. Schwartz	1998	Iliff, CO
Fob257c	*F. oxysporum* f. sp. *betae*	*F. secorum*	H. Schwartz	1998	Brush, CO

[a]Species identification of each isolate at the beginning of studies. *F. oxsporum* f. sp. *betae* isolates were previously reported by Hanson *et al*. (2009) or Hill *et al*. (2011).

[b]Institution of each donor: G. Secor, Department Plant Pathology, North Dakota State University, Fargo, ND, USA; L. Hanson, USDA-ARS, Sugarbeet and Bean Research Unit, East Lansing, MI, USA; H. Schwartz, formerly with Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO, USA.

Phylogenetic analysis

To confirm the identity of isolates included in this study, *TEF1* consensus sequences obtained as described above were used as queries to perform nucleotide database searches using BLASTN (https://blast.ncbi.nlm.nih.gov). *TEF1* sequences of *F. secorum* isolates were added to those previously reported as *F. oxysporum* from sugar beet (Hill *et al.*, 2011; Webb *et al.*, 2012; Covey *et al.*, 2014) for comparison. Sequences were aligned using MUSCLE (Edgar, 2004), and based on Bayesian information criterion scores, MODELFINDER (Kalyaanamoorthy *et al.*, 2017) identified the optimal model of molecular evolution. The portions of sequences showing ambiguous alignment were excluded from the phylogenetic analysis. Phylogenetic analysis of the *Fusarium* population isolated from sugar beet was performed using a total of 69 *TEF1* sequences, each of 461 bp in length. Support for clades in phylogenetic trees was assessed using 1000 maximum-likelihood bootstrap pseudoreplicates of the *TEF1* dataset in IQ-TREE (Chernomor *et al.*, 2016; http://www.iqtree.org/). An additional 53 *TEF1* sequences of the broader *Fusarium* population (obtained from whole genome sequences published in GenBank that were depicted by NCBI as representative genomes; Table S1) were then combined with the 69 *TEF1* sequences from the sugar beet *Fusarium* population to construct another phylogenetic tree representing a larger group of the *Fusarium* genus. For all analyses, *TEF1* sequences of two species in the *F. newnesense* species complex, *F. newnesense* RBG610/NRRL66241 (GenBank KP083261) and *Fusarium* sp. NRRL 25184 (GenBank AF008514), were used to root the phylogeny in MEGA 7 software based on more inclusive analyses (O'Donnell *et al.*, 2013; Laurence *et al.*, 2016).

Table 2 List of sugar beet germplasm or lines used for virulence screening.

Germplasm/cultivar	Information on lines	Donor[a]
USH20	Fusarium susceptible line	L. Panella
FC716	Fusarium susceptible line	L. Panella
Monohikori	Universal susceptible	L. Panella
VDH46177	Fusarium susceptible	SesVanderhave
902735	Fusarium susceptible	SesVanderhave
SYN07064964	Fusarium yellows susceptible	Syngenta-Hilleshog

[a]Institution of each seed donor: L. Panella, formerly with USDA-ARS, 1701 Centre Ave., Fort Collins, CO, USA; SesVanderhave, 5908 52nd Ave. South, Fargo, ND, USA; Syngenta-Hilleshog, 1020 Sugarmill Rd., Longmont, CO, USA.

Sugar beet inoculations

Six susceptible sugar beet lines/germplasm were provided by the breeding programmes of Dr L. Panella (USDA-ARS, Fort Collins, CO, USA), Syngenta-Hilleshog (Longmont, CO, USA), and SesVanderhave USA (Fargo, ND, USA; Table 2). Sugar beet seed were planted into 6.5 cm black plastic cone-shaped containers (Cone-tainers) using pasteurized potting soil (Farfad Professional Growing Mix; Sungro Horticulture) with 1 seed per Cone-tainer. Plants were grown in a greenhouse with an average daytime temperature of 27 °C, 50% relative humidity, and a 16 h photoperiod for 4–5 weeks. Plants were inoculated at the 2- to 3-leaf stage by removing the plants from Cone-tainers, rinsing off as much soil as possible, then dipping the root into a spore suspension of 10^4 conidia mL^{-1} for 5 min with gentle agitation. After inoculation, plants were replanted into new Cone-tainers containing premoistened pasteurized potting soil as described above (Hanson & Hill, 2004; Hanson *et al.*, 2009; Burlakoti *et al.*, 2012; Secor *et al.*, 2014). Plants were inoculated with multiple isolates of *F. secorum* that had been collected from the Red River Valley as well as the type strain *F. secorum* (670-10; Secor *et al.*, 2014; Table 1). Three previously described *F. oxysporum* f. sp. *betae* isolates, F19, Fob257c and Fob220a, were used as positive controls for fusarium yellows (Hanson *et al.*, 2009; Hill *et al.*, 2011). Distilled water was used as the negative (mock) control. Five plants were inoculated per isolate, which were placed in a randomized split-plot design in a greenhouse maintained at 27 °C, 50% relative humidity and a 16 h photoperiod with variety as main plot and isolate as a subplot. Disease symptoms were evaluated on a weekly basis for 4 weeks after inoculation. Fusarium yellowing decline symptoms were evaluated using a modified 0–5 fusarium yellows disease severity rating (Hanson *et al.*, 2009). The area under the disease progress curve (AUDPC; Campbell & Madden, 1990) was calculated for the 4-week period and significant differences compared using PROC MIXED in the SAS statistical program based on a randomized complete block scheme (SAS Institute). The entire experiment was repeated four times.

Table 3 Morphological characteristics of *Fusarium* isolates used in virulence and phylogenetic assays.

		Microconidia		Phialides			Macroconidia			
Isolate	Pigment	Shape	Septate	Length	Type	Heads/chains	Shape	Septate	Chlamydospores	Circinate mycelia
F19	Purple	Oval/reniform	0	Short	Mono	Heads	Falcate	3	Present	—
Fob257c	Salmon (faint)	Oval	0–2	Short	Mono	Heads	Slender, straight	4	Present	Present
Fob220a	White	Oval/reniform	1	Short	Mono	Heads	Falcate	3–4	—	—
784-24-2C	Salmon	Oval	0–2	Short	Mono	Heads	Slender, straight	3–4	—	Present
670-10	Salmon	Oval	0–2	Short	Mono	Heads	Slender, straight	3–4	Present	Present
938-4	White	Oval	0–2	Short	Mono	Heads	Slender, straight	3–4	—	Present
938-6	Salmon (faint)	Oval	0–1	Short	Mono	Heads	Slender, straight	3–4	—	Present
845-1-18	Salmon	Oval	0–2	Short	Mono	Heads	Slender, straight	4	Present	Present
742-28	White	Oval	0	Short	Mono	Heads	Slender, straight	3	—	Present
1090-4-2	Salmon	Oval	2	Short	Mono	Heads	Falcate	4–5	Abundant	Sparse

Morphological characteristics were described after plating on potato dextrose agar (PDA), 1/2 × PDA and carnation leaf agar, performed according to Leslie & Summerell (2006).

Results

Morphological identification of *F. secorum* and previously described *F. oxysporum* isolates from sugar beet

Prior to starting virulence studies, each isolate used for pathogenicity testing was grown on semiselective media to help confirm their identity as either *F. secorum* or *F. oxysporum* (Table 3). A primary observation from these findings was that there was substantial overlap in the morphological traits for each isolate included in this study, making it difficult to differentiate between isolates. Putative *F. secorum* isolates 670-10, 784-24-2C, 938-4, 938-6, 845-1-18 and 742-28 all had morphological characteristics previously described for *F. secorum* including salmon (or white) pigment on PDA, the presence of chlamydospores, 0–2-septate oval microconidia, 3–4-septate macroconidia, and the presence of circinate mycelia (Table 3; Secor *et al.*, 2014). Interestingly, isolate Fob257c, previously designated as *F. oxysporum* f. sp. *betae* (Hill *et al.*, 2011), had salmon-coloured pigments on PDA and exhibited circinate mycelia (Fig. 1) typical of *F. secorum*. Fob220a had white mycelia on PDA, oval/reniform 1-septate microconidia, and 3–4-septate macroconidia. In contrast, isolate F19 had purple pigment on PDA, 0-septate oval/reniform microconidia, falcate 3-septate macroconidia, and did not have circinate mycelia. Finally, isolate 1090-4-2 had salmon pigments and exhibited long, primarily 2-septate, oval microconidia, 4–5-septate macroconidia, abundant chlamydospores and sparse circinate hyphae (Table 3).

TEF1 identification of *Fusarium* isolates from sugar beet

Because growth on semiselective media did not confirm species designations, *TEF1* sequence data for all strains used in this study were generated (Table S1). The *TEF1* sequences of the five *F. secorum* isolates (784-24-2C, 938-4, 938-6, 845-1-18 and 742-28) were 100% identical to each other and to the ex-type strain of *F. secorum* (GenBank accession KX858823.1; Secor *et al.*, 2014). Although Fob257c and Fob220a were previously reported as *F. oxysporum* (Hanson *et al.*, 2009; Hill *et al.*, 2011), the *TEF1* sequences from each isolate were 100% identical to *F. secorum* isolates and results of the BLASTN queries also indicated they were *F. secorum*.

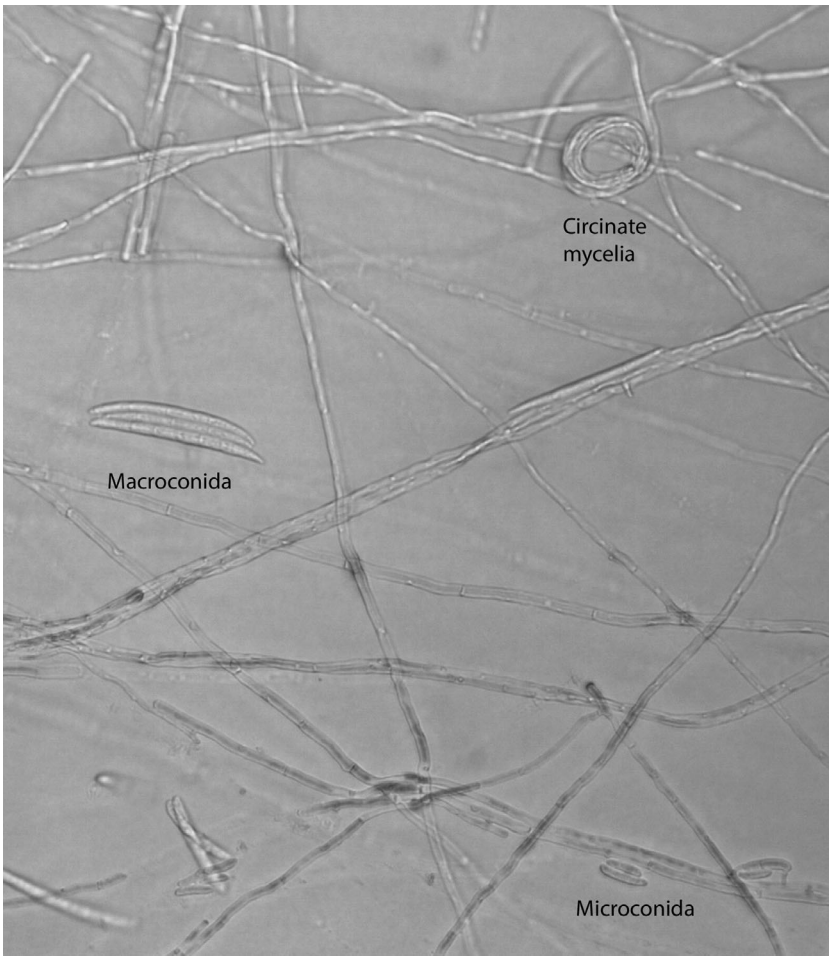

Figure 1 Morphology of *Fusarium* isolate Fob257c grown on carnation leaf agar. Fob257c was previously described as *Fusarium oxysporum* f. sp. *betae* but its colony morphology is similar to *F. secorum* (Secor *et al.*, 2014).

Interestingly, a BLASTN search of GenBank using the *TEF1* sequence of isolate F19 indicated that it is *F. commune* (100% similarity to *F. commune* isolate 9447J #DQ016244.1) and not *F. oxysporum* as previously reported (Hill *et al.*, 2011; Webb *et al.*, 2012; Covey *et al.*, 2014).

Characterization of genetic diversity of *Fusarium* from sugar beet using *TEF1*

Because the data conflicted in part with past species designations, it was decided to add the newly identified *F. secorum TEF1* sequence data to a previously reported phylogenetic tree; it was expected that a new clade would be formed distinct from previously described *F. oxysporum* f. sp. *betae* strains (Hill *et al.*, 2011; Webb *et al.*, 2012; Covey *et al.*, 2014). For these analyses, the MODELFINDER feature in IQ-TREE identified TNe as the optimal model for molecular evolution of the phylogeny of fusaria associated with sugar beet infection (Fig. 2). When these species were included with a broader range of fusaria, MODELFINDER identified TNe+G4 as the optimal model of molecular evolution (Fig. S1). The fusaria from sugar beet were grouped into three groups: *F. nisikadoi* species complex (FNSC), *F. oxysporum* species complex (FOSC), and the *F. fujikuroi* species complex (FFSC; Figs 2 & S1), which were previously named as clades A, C and B, respectively (Hill *et al.*, 2011; Webb *et al.*, 2012; Covey *et al.*, 2014). All *F. secorum* isolates from this study grouped into FFSC containing what had previously been reported as *F. oxysporum* group B, including Fob257c and Fob220a isolates from this study (Fig. 2). Isolate F19 grouped with other isolates reported as *F. commune* associated with the FNSC (Figs 2 & S1). All the other *F. oxysporum* isolates grouped in FOSC (Fig. 2). Phylogenetic analysis of these 69 isolates, along with *TEF1* sequences from multiple fusaria published in GenBank, confirmed that several pathogenic fungal isolates that had been named as *F. oxysporum* f. sp. *betae* were misidentified and they are distributed in phylogenetic groups other than FOSC (Fig. S1).

Fusarium secorum virulence to sugar beet

Because *Fusarium* pathogens of sugar beet have reported genotype by cultivar interactions (Ruppel, 1991; Panella & Lewellen, 2005; Hanson *et al.*, 2009), the relative virulence of each isolate was tested on six susceptible sugar beet cultivars over a 4-week period. Sugar beet cultivars reacted differently to inoculation with different *Fusarium* isolates, with some lines having more severe disease symptoms than others (Fig. 3). For example, *F. secorum* isolates varied in their virulence to sugar beet and the relative ranking of each isolate was dependent on sugar beet genotype (Fig. 3). In general, the most susceptible cultivar in these tests was VDH46177 (Fig. 3f; AUDPC = 16.0; $P < 0.0001$) with the least susceptible variety being USH20 (Fig. 3e; AUDPC = 9.3; $P = 0.0008$). Some isolates were generally more virulent than the others on all varieties tested, whereas other isolates varied in their relative virulence and in onset of disease symptoms according to sugar beet cultivar. For example, isolate F19 was significantly more virulent on line 902735 whereas it had a much smaller AUDPC on USH20, similar to several other isolates on that line (Fig. 3a,e). In another example, on sugar beet line FC716, most isolates tested caused similar amounts of disease (Fig. 3b), whereas there were clear differences in their relative virulences on other lines such as 902735 or SYN07064964 (Fig. 3a,d). Isolate 742-28 was virulent on some lines but not on others (Fig. 3). Overall, isolates F19, Fob220a, 670-10 and 784-24-2C were all highly virulent (Fig. 4) and would completely kill sugar beet plants, regardless of cultivar, by 4 weeks (Fig. 4). Moderately virulent isolates included 845-1-18, 938-4, 938-6 and Fob257c. Only one isolate, 742-28, was considered weakly virulent because it caused only minor symptoms independent of the cultivar in question (Fig. 4). Finally, 1090-4-2 was nonpathogenic because it was not statistically different from the negative control (Fig. 4).

Discussion

Fusarium species are among the most diverse and prevalent of fungal plant pathogens (Ma *et al.*, 2010). Species of *Fusarium* were traditionally defined by morphological characteristics (Nelson *et al.*, 1981); however, multilocus molecular systematic studies have revealed that *Fusarium* comprises at least one order of magnitude more species than the 30 reported in Nelson *et al.* (1983). O'Donnell *et al.* (2015) reviewed the status of the molecular identification of fusaria and recommended that *TEF1* worked well for estimating species diversity within the genus. As presently defined, *Fusarium* encompasses 23 species complexes and nine monotypic lineages (Laurence *et al.*, 2011; Geiser *et al.*, 2013; O'Donnell *et al.*, 2013; Sandoval-Denis *et al.*, 2018).

Multiple *Fusarium* spp. (Hanson & Hill, 2004; Burlakoti *et al.*, 2012) have been implicated in yield and sucrose losses in sugar beet since the first report of the disease fusarium yellows (Stewart, 1931). While many *Fusarium* species have been reported to cause disease, *F. oxysporum* f. sp. *betae* has been considered the primary causal agent based on the prevalence of isolations from infected sugar beets and aggressiveness of isolates in pathogenicity testing (Stewart, 1931; Ruppel, 1991; Hanson & Hill, 2004). Significant work by others previously characterized the genetic diversity of *Fusarium* from sugar beet into three groups designated at the time as A, B and C (Hill *et al.*, 2011; Webb *et al.*, 2012; Covey *et al.*, 2014). A primary finding from the present study was that some isolates previously reported as *F. oxysporum* f. sp. *betae* (Hill *et al.*, 2011; Webb *et al.*, 2012; Covey *et al.*, 2014) are actually *F. secorum*. Thus, significantly, *F. secorum* isolates have apparently been isolated from sugar beet prior to the first reports of

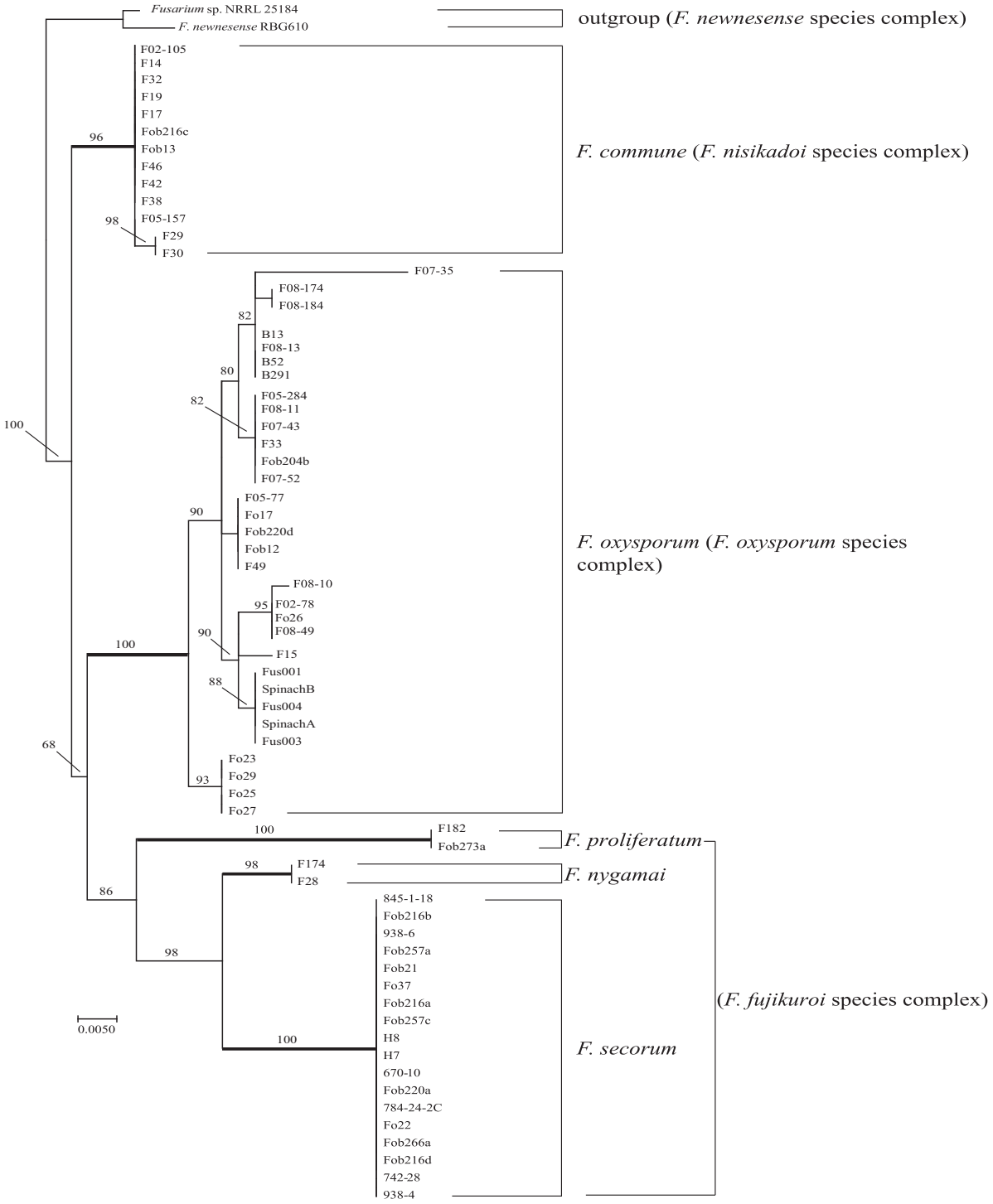

Figure 2 Maximum-likelihood (ML) phylogeny of fusaria associated with sugar beet showing disease symptoms, based on analysis of *TEF1* sequences (461 bp alignment). Numbers above nodes represent ML bootstrap (BS) based on 1000 pseudoreplicates of the data. The ML-BS analysis was conducted with IQ-TREE using TNe as the optimal model of molecular evolution. The phylogeny was rooted on sequences of two species in the *Fusarium newnesense* species complex, based on more inclusive analyses (O'Donnell *et al.*, 2013).

Rivera *et al.* (2008) and Secor *et al.* (2014). These isolates, initially reported as *F. oxysporum* and now identified as *F. secorum*, were originally collected between 1998 and 2004 and largely from Colorado, although some were collected from sugar beet in other production regions (Hill *et al.*, 2011). Symptoms of fusarium yellows

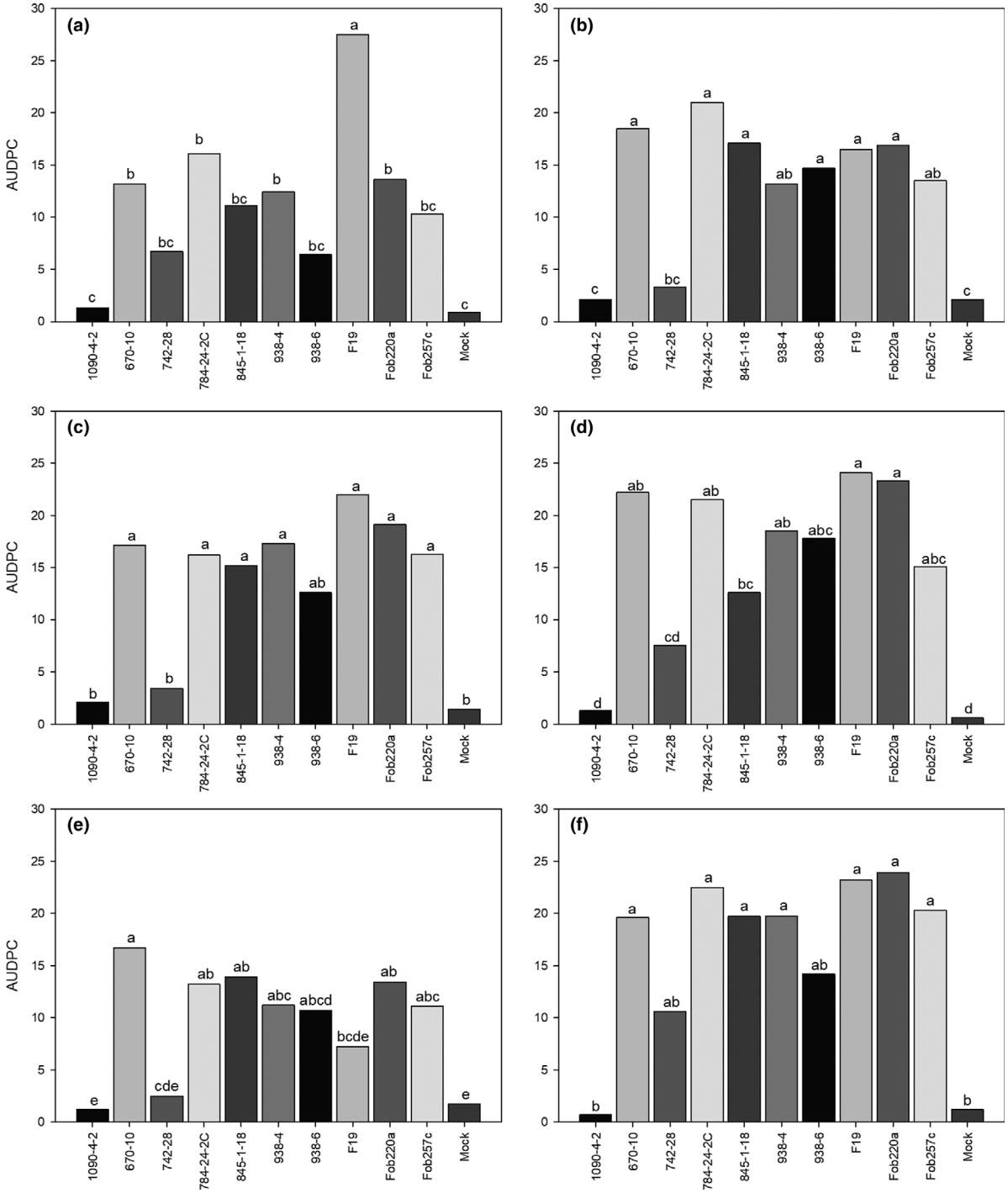

Figure 3 Disease severity, measured as area under the disease progress curve (AUDPC), for each *Fusarium secorum* isolate, compared to standard fusarium yellows test isolates (F19, Fob257c and Fob220a) on six susceptible sugar beet lines with differing genetic backgrounds: (a) 902735, (b) FC716, (c) Monohikori, (d) SYN07064964, (e) USH20, (f) VDH46177. Different letters above bars indicate statistically significant differences in disease severity at $P < 0.05$.

caused by *F. oxysporum* f. sp. *betae* have traditionally included wilting, interveinal yellowing (which usually starts with older leaves and gradually leads to leaf death) and internal brown to grey-brown vascular discoloration (Hanson & Hill, 2004). However, a half-leaf yellowing has also been reported (Windels *et al.*, 2005; Hanson *et al.*, 2009) that is also typical of fusarium yellowing decline (Secor *et al.*, 2014). Given these reported

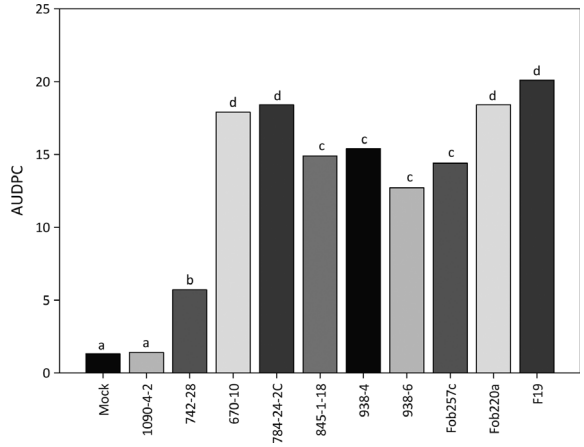

Figure 4 Mean disease severity, measured as area under the disease progress curve (AUDPC), for each *Fusarium secorum* isolate compared to standard fusarium yellows test isolates (F19, Fob257c and Fob220a). The data presented is based on four experimental replications. Different letters above bars indicate statistically significant differences in virulence of isolates at $P < 0.05$.

differences in disease phenotypes, it is quite possible that *F. secorum* was prevalent throughout the major sugar beet production areas prior to the first report of fusarium yellowing decline (Rivera *et al.*, 2008; Secor *et al.*, 2014).

Another major finding from the present study is that some isolates that were initially reported as *F. oxysporum* are *F. commune*. The latter species is closely related to both the FOSC and FFSC (O'Donnell *et al.*, 2013), with morphological characters very similar to *F. oxysporum* except for the presence of polyphialides (Skovgaard *et al.*, 2003), which are lacking in *F. oxysporum*. *Fusarium commune* has been reported to be pathogenic on pea (*Pisum sativum*), white pine (*Pinus monticola*) and Douglas-fir (*Pseudotsuga menziesii*) (Skovgaard *et al.*, 2003; Stewart *et al.*, 2006) but has not been reported on sugar beet. Additional pathogenicity testing using a larger collection of *F. commune* isolates should be performed in the future.

Finally, the findings of this study suggest that weakly virulent or nonpathogenic isolates of *Fusarium* from sugar beet, previously associated with group C (Hill *et al.*, 2011; Webb *et al.*, 2012; Covey *et al.*, 2014), are mostly grouped in the FOSC and remain identified as *F. oxysporum*. Because these FOSC isolates are or weak or nonpathogens of sugar beet (Hill *et al.*, 2011; Webb *et al.*, 2012), none were included in the pathogenicity testing.

To elucidate further the prevalence and importance of the *Fusarium* species discovered in the current investigation, new pathogen surveys of sugar beet production regions should be undertaken. Having a strong understanding of the diversity of *Fusarium* spp. that cause disease on sugar beet will be important for regional management recommendations for each species, screening for resistance, and the development of rapid diagnostic tools. In this study, it has been shown that *F. secorum* isolates vary in virulence to sugar beet and that this can be influenced by sugar beet genotype. Likewise, Hanson *et al.* (2009) reported that fusarium disease severity varied according to both sugar beet genotype and pathogen isolate. Burlakoti *et al.* (2012) suggested that isolates of *F. secorum* were more virulent than other sugar beet *Fusarium* pathogens. Therefore, the present findings stress the importance of testing sugar beet lines with *Fusarium* isolates that encompass the whole range of virulence to ensure that cultivars with broad-based resistance to phytopathogenic fusaria are developed.

In particular, the findings suggest that screening with *F. secorum* may be particularly relevant because it is now likely that this pathogen has a larger geographical range than previously understood. Such studies would further characterize the diversity of *F. secorum*, offer genetic markers to identify pathogenic isolates and ultimately provide insight into host susceptibility and resistance.

Acknowledgements

The authors would like to thank Dr Kerry O'Donnell with USDA-ARS-NCAUR in Peoria, IL, USA for editorial comments and gracious assistance with phylogenetic analysis. They would also like to thank Addison Reed and Nicholas Metz for assistance in preliminary sequence analysis and pictures for the morphological identification of some of the isolates. Finally, the authors thank Roshan Sharma Poudel for technical assistance in writing the code to extract *TEF1* from genomic sequences in GenBank. Additional funding for this project was provided by the Sugar Beet Research and Extension Board of Minnesota/North Dakota. R. de Jonge was financially supported by an EMBO Long-Term Fellowship (ALTF 359-2013) and a postdoctoral fellowship of the Research Foundation Flanders (FWO 12B8116N). Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

Data Availability Statement

The data that support the findings of this study are openly available in GenBank at www.ncbi.nlm.nih.gov/genbank/, accession numbers MH926020–MH926026.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Figure S1. Maximum likelihood (ML) phylogeny of fusaria associated with sugar beet showing disease symptoms and a broader *Fusarium* population from GenBank, based on analysis of *TEF1* sequences (461 bp alignment). Numbers above nodes represent ML bootstrap (BS) based on 1000 pseudoreplicates of the data. The ML-BS analysis was conducted with IQ-TREE using TNe + G4 as the optimal model of molecular evolution. The phylogeny was rooted on sequences of two species in the *F. newnesense* species complex based on more inclusive analyses (O'Donnell *et al*.,). All fusaria associated with sugar beet infection are indicated by bold letters.

Table S1. Citation and/or GenBank accession numbers for sources of *TEF1* sequences of isolates included in phylogenetic trees.