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

K. M. Webb\textsuperscript{a*†}, S. Shrestha\textsuperscript{b*c†}, R. Trippe III\textsuperscript{a}, V. Rivera-Varas\textsuperscript{c}, P. A. Covey\textsuperscript{a}, C. Freeman\textsuperscript{a}, R. de Jonge\textsuperscript{d}, G. A. Secor\textsuperscript{c} and M. Bolton\textsuperscript{b*c}

\textsuperscript{a}United States Department of Agriculture – Agricultural Research Service (USDA-ARS), Soil Management and Sugar Beet Research Unit, 1701 Centre Ave., Fort Collins, CO, 80526; \textsuperscript{b}USDA-ARS, Red River Valley Agricultural Research Center; \textsuperscript{c}Department of Plant Pathology, North Dakota State University, Fargo, ND, 58108, USA; and \textsuperscript{d}Plant-Microbe Interactions, Department of Biology, Science4Life, Utrecht University, 3584 CH Utrecht, Netherlands

*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;
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., 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 isolates of *F. secorum* et al. f. sp. *betae*

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.

### 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.

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

| Isolate name | Species | Species ID after studies | Donor | 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 |
| Feb220a      | *F. oxysporum* f. sp. *betae* | *F. secorum* | H. Schwartz | 1996 | Iliff, CO |
| Feb237c      | *F. oxysporum* f. sp. *betae* | *F. secorum* | H. Schwartz | 1996 | Brush, CO |

*a*Species identification of each isolate at the beginning of studies. *F. oxysporum* 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. 

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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. secomorum* 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).

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 seedlings 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 spor suspension of 10⁴ 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. secomorum* that had been collected from the Red River Valley as well as the type strain *F. secomorum* (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 variation 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 2 List of sugar beet germplasm or lines used for virulence screening.

| Germplasm/cultivar | Information on lines | Donor* |
|-------------------|----------------------|--------|
| USH20             | Fusarium susceptible line | L. Panella |
| FC718             | Fusarium susceptible line | L. Panella |
| Monohikori        | Universal susceptible | L. Panella |
| VDH46177          | Fusarium susceptible | SesVanderhave |
| 902735            | Fusarium susceptible | SesVanderhave |
| SYN07064964       | Fusarium yellows susceptible | Syngenta-Hilleshog |

*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.

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

| Isolate | Pigment | Microconidia | Phialides | Macroconidia | Chlamydospores | Circinate mycelia |
|---------|---------|--------------|-----------|--------------|----------------|------------------|
| F19     | Purple  | Oval/reiform | 0         | Short Mono Heads | Falcate | 3 | Present | — |
| Fob257c | Salmon (taint) | Oval | 0–2      | Short Mono Heads | Slender, straight | 4 | Present | Present |
| Fob220a | White | Oval/reiform | 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 |
| 938-4   | White  | Oval | 0–2      | Short Mono Heads | Slender, straight | 3–4 | — | Present |
| 938-6   | Salmon (taint) | Oval | 0–1     | Short Mono Heads | Slender, straight | 3–4 | — | Present |
| 845-1-18 | Salmon | Oval | 0–2      | Short Mono Heads | Slender, straight | 3 | — | Present |
| 742-28  | White  | Oval | 0        | Short Mono Heads | Falcate | 4–5 | Abundant | Sparse |
| 1000-4-2 | Salmon | Oval | 2        | Short Mono Heads | Falcate | — | — | — |

Morphological characteristics were described after plating on potato dextrose agar (PDA), 1/2 x 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 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; Fanella & 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...
Rivera et al. (2008) and Secor et al. (2014). These isolates, initially reported as *F. oxysporum* and now identified as *F. securum*, 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...
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

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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 $P < 0.05$. 

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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.

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**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 TN+G4 as the optimal model of molecular evolution. The phylogeny was rooted on sequences of two species in the F. neoamurense 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.