Comparative Pathogenicity and Virulence of *Fusarium* Species on Sugar Beet

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Abstract

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In all, 98 isolates of three *Fusarium* spp. (18 *Fusarium oxysporum*, 30 *F. graminearum*, and 50 *Fusarium* sp. nov.) obtained from sugar beet in Minnesota were characterized for pathogenicity and virulence on sugar beet in the greenhouse by a bare-root inoculation method. Among the 98 isolates tested, 80% of isolates were pathogenic: 83% of the *F. oxysporum* isolates, 57% of the *F. graminearum* isolates, and 92% of the *Fusarium* sp. nov. isolates. Symptoms varied from slight to moderate wilting of the foliage, interveinal chlorosis and necrosis, and vascular discoloration of the taproot without any external root symptoms. Among the pathogenic isolates, 14% were highly virulent and

12% were moderately virulent. Most of the highly virulent isolates (91%) and moderately virulent isolates (89%) were *Fusarium* sp. nov. All pathogenic isolates of *F. graminearum* and most pathogenic isolates (87%) of *F. oxysporum* were less virulent. In general, more-virulent isolates induced first foliar symptoms earlier compared with less-virulent isolates. This study indicates that both *F. oxysporum* and *Fusarium* sp. nov. should be used in greenhouse and be present in field studies used for screening and developing sugar beet cultivars resistant to Fusarium yellows complex for Minnesota and North Dakota.

Minnesota and North Dakota produce more than 50% of the sugar beet (*Beta vulgaris* L.) in the United States (15). The sugar beet industry in the bi-state area contributes over \$3 billion in total economic activities (1). Fusarium yellows, a devastating disease of sugar beet, has become a serious problem for growers, especially in the Moorhead factory district in Minnesota (10,19,32). Fusarium yellows was first described by Stewart in 1931 from sugar beet samples collected in Colorado (39). The causal pathogen initially was identified as *Fusarium congruatum* var. *betae* D. Stewart and subsequently renamed *F. oxysporum* f. sp. *betae* W.C. Snyder & H.N. Hansen (38). Six different *Fusarium* spp. (*F. oxysporum*, *F. acuminatum* Ellis & Everh., *F. avenaceum* (Fr.) Sacc., *F. solani* (Mart.) Appel & Wollenw., *F. moniliforme* J. Sheld., and *F. graminearum* Schwabe) have been reported to cause Fusarium yellows in the United States (16,17,23,34). However, *F. oxysporum* f. sp. *betae* is considered to be the primary causal organism of Fusarium yellows (19). Rivera et al. (33) reported *Fusarium* sp. nov. also as a causal agent of yellowing decline in sugar beet. All of these *Fusarium* spp. cause similar symptoms on sugar beet (17).

Fusarium yellows is characterized by interveinal chlorosis and wilting as foliar symptoms, and vascular discoloration of the taproot with no external necrosis of the taproot or secondary roots (39). The disease symptoms first appear on the oldest leaf as interveinal chlorosis followed by necrosis (2). As the disease progresses, the pathogen may affect several whorls of leaves and the leaves may change from yellow to a brown necrotic color. Sometimes sectoring occurs, with only one side of the leaf along the midrib turning yellow, followed later by the entire leaf. Damping-off and root rot occur on sugar beet but seed-producing beet may also be affected by stalk blight (14,28). Fusarium yellows causes significant quantitative and qualitative yield losses due to reduced plant population, poor growth of plants, and increased impurities in the extracted juice (19,20). As an added concern, Khan et al. (27)

found increased respiration rate and reduced sucrose concentration of infected sugar beet roots compared with healthy roots after storage.

Fusarium yellows was reported in the Red River Valley of Minnesota and North Dakota during 2002 (26) and the disease was confirmed in 2005 (41). Growers of the American Crystal Sugar Company, the largest sugar beet cooperative in the United States, (25) are concerned about Fusarium yellows because the disease is now present in all their growing areas, especially the Moorhead Factory District. Rivera et al. (33) isolated *F. oxysporum*, *F. graminearum*, and *Fusarium* sp. nov. from sugar beet in fields located in the Moorhead Factory District in Minnesota. The objective of this study was to characterize both the pathogenicity and virulence of isolates of these three *Fusarium* spp. on a known Fusarium yellows-susceptible cultivar of sugar beet in the greenhouse. The pathogenicity and virulence of *Fusarium* sp. nov. likely has not been well studied or documented because most research has been done with *F. oxysporum* f. sp. *betae*.

Materials and Methods

Plant material and growing conditions. Fusarium yellows-susceptible 'Van der Have 46177' (31) was grown in the greenhouse. Seed were sown in cone-tainers (3.8 cm in diameter by 21 cm deep; Stuewe & Sons, Inc., Corvallis, OR) filled with Sunshine Mix 1 (Sun Gro Horticulture, Bellevue, WA) amended with 200 g of Osmocote 14-14-14 (Scotts Company, Marysville, OH) per 0.76 m³ of Sunshine Mix 1. Two seeds were sown in each cone-tainer at a depth of 2.2 cm. Planting was done during the last week of November 2006 and mid-January 2007. Less vigorous plants were identified and removed 10 days after seedling emergence, leaving one plant per cone-tainer. Plants were grown in the greenhouse under fluorescent light set and maintained for a 16-h photoperiod and temperature set and maintained at 27 ± 2°C.

Isolates and inoculum preparation. *Fusarium* isolates were obtained from infected sugar beet from fields at Sabin and Georgetown, Clay County, and Fossum, Norman County in Minnesota in 2005. The Sabin and Georgetown sites were sugar beet screening nurseries and the Fossum site was a commercial field. Isolates were recovered and identified using standard procedures as described by Nelson et al. (30), including growth characteristics on potato dextrose agar (PDA), conidia morphology, and chlamydo-

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spore formation on carnation leaf agar. Isolates were stored on PDA in tubes at -80°C until required (35). In this study, 18 isolates of *F. oxysporum*, 30 isolates of *F. graminearum*, and 50 isolates of *Fusarium* sp. nov. were used (Table 1). All isolates were transferred to freshly prepared half-strength (H)PDA (5) and grown for 10 days at 25 to 26°C with a 12-h photoperiod provided by fluorescent light. The plates were flooded with sterile distilled water and scraped with a sterile glass rod to obtain fungal mycelia and conidia. Conidia and mycelia were separated by filtering the suspension through cheesecloth. Conidia concentration was adjusted to 4×10^4 macroconidia ml^{-1} using a hemocytometer. Two drops of polyoxyethylene-20-sorbitan monolaurate (Tween 20; Sigma-Aldrich, St. Louis) was added to 100 ml of distilled water before autoclaving to help even distribution and adherence of inoculum.

Inoculation and disease assessment. Ninety-eight isolates were used to inoculate 38-day-old sugar beet plants at the eight-leaf growth stage. Plants were carefully removed from cone-tainers, and roots were washed with sterilized water and then dipped in a suspension of *Fusarium* conidia for 8 min, as described by Hanson and Hill (17). The suspension was thoroughly shaken every minute for uniform distribution of conidia. Roots were dipped in sterile

distilled water as a control. Plants were then transplanted immediately to cone-tainers filled with fresh Sunshine Mix 1, incubated in a growth chamber at $21 \pm 2^{\circ}\text{C}$ for 2 days to reduce transplanting shock, and then transferred into a greenhouse at $27 \pm 2^{\circ}\text{C}$. Natural light was supplemented with fluorescent light for a photoperiod of 16 h day^{-1} .

To avoid confusion with transplanting shock, the oldest two leaves were removed 3 days after transplanting. Plants were carefully hand watered daily to avoid splashing of soil or water between different treatments. The experiment was designed as a randomized complete block with five replicates, where a single plant served as the experimental unit. The experiment was conducted in 2006 and repeated in 2007. A preliminary study (*data not shown*) indicated that some isolates did not cause vascular discoloration until 35 days after inoculation (DAI). As such, inoculated plants were evaluated for 60 DAI in this study. Plants were independently rated twice weekly by the same rater for foliar symptoms using a disease severity rating scale of 0 to 4 similar to Hanson and Hill (17), with slight modifications, where 0 = healthy plants with no symptoms; 1 = plants stunted and wilted, with few yellowed leaves (1 to 24%); 2 = chlorosis and necrosis of leaves (25 to 59%); 3 =

Table 1. Days to first foliar symptoms, area under the disease progress curve (AUDPC), median disease severity (DS), relative treatment effect on disease severity (P_{ij}), along with confidence intervals for relative treatments effect of 78 *Fusarium* isolates on the susceptible sugar beet 'Van der Have 46177' in greenhouse studies conducted in 2006 and 2007

Isolate number	Species	Days ^v	AUDPC	Median	Disease severity ^u	
					Treatment relative effect	
					(P_{ij}) ^w	95% CI ^x
525	<i>Fusarium</i> sp. nov.	13 D	3,692 a	4	0.939	0.932–0.946
408	<i>Fusarium</i> sp. nov.	15 B–D	2,387 de	4	0.925	0.904–0.941
605	<i>Fusarium</i> sp. nov.	22 v–x	2,498 de	4	0.925	0.904–0.941
581.52	<i>Fusarium</i> sp. nov.	19 y–A	2,832 cd	4	0.912	0.879–0.936
129	<i>Fusarium</i> sp. nov.	16 A–D	2,926 b–d	4	0.912	0.879–0.936
229b	<i>Fusarium</i> sp. nov.	14 CD	3,507 ab	4	0.913	0.874–0.940
581.508	<i>Fusarium</i> sp. nov.	18 y–A	2,832 cd	4	0.913	0.874–0.940
581.63	<i>Fusarium</i> sp. nov.	19 x–z	2,610 c–e	4	0.888	0.841–0.921
581.520	<i>Fusarium</i> sp. nov.	17 z–C	3,132 a–c	4	0.829	0.637–0.928
104	<i>Fusarium</i> sp. nov.	24 s–w	2,064 ef	4	0.851	0.712–0.928
581.513	<i>Fusarium</i> sp. nov.	19 x–z	2,220 ef	2	0.862	0.816–0.897
628	<i>Fusarium</i> sp. nov.	17 z–C	2,181 ef	2	0.848	0.812–0.877
630	<i>Fusarium</i> sp. nov.	15 B–D	1,635 fg	2	0.835	0.806–0.860
581.307	<i>Fusarium</i> sp. nov.	21 w–y	1,374 gh	2	0.751	0.611–0.852
581.202	<i>Fusarium</i> sp. nov.	18 y–B	1,137 g–i	2	0.689	0.550–0.801
210	<i>Fusarium</i> sp. nov.	27 m–r	788 i–k	2	0.641	0.450–0.761
428	<i>Fusarium</i> sp. nov.	27 m–r	482 j–m	2	0.641	0.5–0.761
518	<i>Fusarium</i> sp. nov.	27 m–s	788 i–k	2	0.641	0.5–0.761
581.203	<i>Fusarium</i> sp. nov.	25 r–v	228 k–m	1	0.387	0.381–0.393
528.401	<i>Fusarium</i> sp. nov.	32 d–i	223 k–m	1	0.387	0.381–0.393
581.21	<i>Fusarium</i> sp. nov.	33 d–g	179 m	1	0.387	0.381–0.393
581.222	<i>Fusarium</i> sp. nov.	37 a–c	179 m	1	0.387	0.381–0.393
581.228	<i>Fusarium</i> sp. nov.	30 g–l	214 k–m	1	0.387	0.381–0.393
581.302	<i>Fusarium</i> sp. nov.	30 h–m	193 m	1	0.387	0.381–0.393
581.506	<i>Fusarium</i> sp. nov.	25 r–v	218 k–m	1	0.387	0.381–0.393
581.516	<i>Fusarium</i> sp. nov.	28 l–q	201 lm	1	0.387	0.381–0.393
581.525	<i>Fusarium</i> sp. nov.	33 c–f	296 k–m	1	0.387	0.381–0.393
581.528	<i>Fusarium</i> sp. nov.	32 e–j	330 k–m	1	0.387	0.381–0.393
581.62	<i>Fusarium</i> sp. nov.	26 n–t	193 m	1	0.387	0.381–0.393
581.529	<i>Fusarium</i> sp. nov.	28 k–p	386 k–m	1	0.387	0.381–0.393
582.315	<i>Fusarium</i> sp. nov.	24 r–v	219 k–m	1	0.387	0.381–0.393
582.428	<i>Fusarium</i> sp. nov.	26 p–u	210 k–m	1	0.387	0.381–0.393
582.322	<i>Fusarium</i> sp. nov.	28 k–p	349 k–m	1	0.387	0.381–0.393
578.1b	<i>Fusarium</i> sp. nov.	27 m–s	166 m	1	0.387	0.381–0.393
578.1B	<i>Fusarium</i> sp. nov.	25 p–u	219 k–m	1	0.387	0.381–0.393
578.1c	<i>Fusarium</i> sp. nov.	25 p–u	219 k–m	1	0.387	0.381–0.393
578.1d	<i>Fusarium</i> sp. nov.	29 j–o	219 k–m	1	0.387	0.381–0.393

(continued on next page)

^u Symbol: – = data not calculated.

^v Average number of days from inoculation until the first symptoms were observed.

^w Estimated treatment relative effect.

^x Upper–lower values of 95% confidence interval (CI) for treatment relative effect.

^y No symptoms were observed at time the experiment was terminated.

^z LSD = Fisher's protected least significant difference ($P = 0.05$).

crown becoming dried and brown to black in color, leaves dying (60 to 89%); and 4 = death of entire plant (90 to 100%). The isolates producing median disease severity values <1, 1 to 1.9, 2 to 2.9, and 3 to 4 were categorized as nonpathogenic, less virulent, moderately virulent, and highly virulent, respectively. The number of days required for appearance of first foliar symptom was recorded for each isolate. Isolations were made from root, crown, and petiole of at least three plants per treatment as described above to confirm pathogenicity.

Data analyses. Foliar disease severity ratings were converted to percentages using the midpoint rule, and the foliar severity value for each rating category was used to calculate area under the disease progress curve (AUDPC; 8). Median disease severity, days until first symptom detection, and AUDPC were used to characterize virulence of each isolate and species. Regression analysis was conducted to assess the relationship between days required to induce first symptoms and disease severity.

Isolates resulting in a median disease severity of 0 were considered nonpathogenic and were excluded from data analysis for characterization of pathogenic isolates. A homogeneous test was conducted for the two experiments based on the calculated *F* values, as described by Gomez and Gomez (13), before combining the data from the two experiments. Analysis of variance, for the number of

days required for appearance of first symptom, and AUDPC were performed using the Statistical Analysis System (SAS, version 9.1; SAS Institute Inc., Cary, NC). Fisher's protected least significance difference of means at $\alpha = 0.05$ was calculated to compare treatments.

The Kruskal-Wallis test (3) was performed to determine whether the three *Fusarium* spp. differ from each other in terms of disease severity. The test was conducted using the MIXED procedure of SAS with the LSMEANS option (version 9.2; SAS Institute Inc.) (3,36). Additionally, the estimated relative effects of treatments were compared using LD_CI macro (3).

Results

Plants inoculated with distilled water were healthy, with no *Fusarium* yellows symptoms. Among the 98 isolates tested, 80% (78 isolates) were pathogenic and the remaining 20% (20 isolates) were nonpathogenic. In all, 92% (46 of 50) of the *Fusarium* sp. nov. isolates, 83% (15 of 18) of the *F. oxysporum* isolates, and 57% (17 of 30) of the *F. graminearum* isolates were pathogenic based on the median value of the disease severity and relative effect of disease severity compared with the noninoculated control. Among the pathogenic isolates, 22% (10 of 46) of *Fusarium* sp. nov. and 7% (1 of 15 isolates) of *F. oxysporum* were highly virulent (sever-

Table 1. (continued from preceding page)

Isolate number	Species	Days ^v	AUDPC	Median	Disease severity ^u	
					Treatment relative effect	
					(<i>P</i> _{ij}) ^w	95% CI ^x
578.1e	<i>Fusarium</i> sp. nov.	34 c–f	149 m	1	0.387	0.381–0.393
578.2c	<i>Fusarium</i> sp. nov.	33 d–g	360 k–m	1	0.387	0.381–0.393
113	<i>Fusarium</i> sp. nov.	25 q–v	210 k–m	1	0.387	0.381–0.393
229a	<i>Fusarium</i> sp. nov.	23 t–w	210 k–m	1	0.387	0.381–0.393
255	<i>Fusarium</i> sp. nov.	35 b–d	193 m	1	0.387	0.381–0.393
423	<i>Fusarium</i> sp. nov.	31 e–j	309 k–m	1	0.387	0.381–0.393
506	<i>Fusarium</i> sp. nov.	38 a	201 m	1	0.387	0.381–0.393
523	<i>Fusarium</i> sp. nov.	36 a–c	295 k–m	1	0.387	0.381–0.393
23b	<i>Fusarium</i> sp. nov.	36 a–b	291 k–m	1	0.387	0.381–0.393
218	<i>Fusarium oxysporum</i>	19 x–z	2,832 cd	4	0.913	0.874–0.941
581.121	<i>F. oxysporum</i>	15 B–D	2,051 ef	2	0.812	0.694–0.891
581.202	<i>F. oxysporum</i>	22 v–x	635 j–l	1	0.520	0.391–0.647
581.609	<i>F. oxysporum</i>	24 s–w	512 j–m	1	0.472	0.366–0.581
581.205	<i>F. oxysporum</i>	33 d–g	175 m	1	0.387	0.381–0.393
581.205a	<i>F. oxysporum</i>	33 c–g	140 m	1	0.387	0.381–0.393
581.225a	<i>F. oxysporum</i>	23 u–w	254 k–m	1	0.387	0.381–0.393
581.318	<i>F. oxysporum</i>	34 c–e	140 m	1	0.387	0.381–0.393
581.321	<i>F. oxysporum</i>	28 k–p	184 m	1	0.387	0.381–0.393
581.415b	<i>F. oxysporum</i>	24 s–w	131 m	1	0.387	0.381–0.393
581.615	<i>F. oxysporum</i>	31 f–k	210 k–m	1	0.387	0.381–0.393
578.2a	<i>F. oxysporum</i>	26 o–u	184 m	1	0.387	0.381–0.393
578.2b	<i>F. oxysporum</i>	25 q–v	219 k–m	1	0.387	0.381–0.393
578.3d	<i>F. oxysporum</i>	25 r–v	236 k–m	1	0.387	0.381–0.393
609	<i>F. oxysporum</i>	32 e–j	263 k–m	1	0.387	0.381–0.393
581.304	<i>F. graminearum</i>	30 h–m	201 lm	1	0.387	0.381–0.393
581.403	<i>F. graminearum</i>	25 p–u	123 m	1	0.387	0.381–0.393
581.404	<i>F. graminearum</i>	26 p–u	158 m	1	0.387	0.381–0.393
581.523	<i>F. graminearum</i>	32 e–j	369 k–m	1	0.387	0.381–0.393
581.628	<i>F. graminearum</i>	32 e–j	219 k–m	1	0.387	0.381–0.393
581.101	<i>F. graminearum</i>	17 z–C	1,025 h–j	1	0.556	0.422–0.683
578.2c	<i>F. graminearum</i>	28 l–q	149 m	1	0.387	0.381–0.393
578.2e	<i>F. graminearum</i>	23 u–w	236 k–m	1	0.387	0.381–0.393
578.2f	<i>F. graminearum</i>	23 t–w	175 m	1	0.387	0.381–0.393
578.5b	<i>F. graminearum</i>	32 d–h	369 k–m	1	0.387	0.381–0.393
578.5c	<i>F. graminearum</i>	29 i–n	219 k–m	1	0.387	0.381–0.393
578.5d	<i>F. graminearum</i>	27 m–r	193 m	1	0.387	0.381–0.393
578.5D	<i>F. graminearum</i>	31 f–k	386 k–m	1	0.387	0.381–0.393
202	<i>F. graminearum</i>	31 f–k	386 k–m	1	0.387	0.381–0.393
578.1a	<i>F. graminearum</i>	29 h–m	325 k–m	1	0.387	0.381–0.393
578.2e	<i>F. graminearum</i>	29 h–m	175 m	1	0.387	0.381–0.393
47 / 48	<i>F. graminearum</i>	38 ab	295 k–m	1	0.387	0.381–0.393
Noninoculated check ^y	...	...	0	0	0.000	0.000–0.000
Overall mean	...	26.3	754	1.5	–	–
<i>P</i> > <i>F</i>	...	<0.01	<0.01	<0.05	<0.05	0.05
LSD ^z	...	3.1	585	–	–	–

ity rating of 3 to 4; Fig. 1; Table 1). Three of the most virulent isolates of *Fusarium* sp. nov. caused death of the plants. In total, 6% of *F. oxysporum* and 16% of *Fusarium* sp. nov. were moderately virulent (severity rating of 2 to 2.9; Fig. 1). Of the 78 pathogenic isolates, 74% (58 isolates) of the isolates were less virulent and consisted of 48% (28 isolates) *Fusarium* sp. nov., 22% (13 isolates) *F. oxysporum*, and 29% (17 isolates) *F. graminearum* (Fig. 1).

An *F* test for the homogeneity of residual mean squares from the individual analyses of variance was not significant for measurements of disease severity ($P = 0.08$), days required for the first symptom to appear ($P = 0.28$), and AUDPC ($P = 0.33$). Analyses of combining data from the two experiments showed that there was no significant difference in 2006 and 2007 for disease severity ($P = 0.66$), days required for the first symptoms to appear ($P = 0.87$), and AUDPC ($P = 0.98$). The interaction between year and isolate was not significant for disease severity ($P = 0.86$) and AUDPC ($P = 0.97$) but was statistically significant ($P < 0.01$) for the number of days required to induce the first disease symptoms. However, the interaction component accounted for as little as 5.6% of the total variance for the number of days required for the first disease symptom to appear. All these indicated that measurements of *Fusarium* disease metrics using bare-root inoculation and under controlled greenhouse conditions were reproducible and that the ranking of different isolates in pathogenicity and virulence should remain the same regardless of when the evaluation programs are carried out.

The disease severity, AUDPC, and days required for appearance of first symptom were significantly different ($P < 0.05$) among pathogenic isolates considered pathogenic, those which caused disease severities ≥ 1 (Table 1). Generally, more-virulent isolates induced first foliar symptoms earlier compared with less-virulent isolates (Table 1). The regression analysis of combined observations from three different *Fusarium* spp. showed that days required after inoculation for first symptoms accounted for 64% variability in disease severity ($R^2 = 0.64$, $P < 0.01$) between number of days required for first symptom development and disease severity (Fig. 2). The number of days required to induce the first symptom was 13 to 23 for the highly virulent isolates, 15 to 21 for the moderately virulent isolates, and 17 to 38 for the less virulent isolates. Isolate 525 (a *Fusarium* sp. nov.) was the most virulent and induced foliar yellowing symptoms at 13 DAI and death of entire plants by 32 DAI, and also resulted in the highest AUDPC and the highest vascular discoloration of roots. AUDPC caused by pathogenic

isolates varied from 123 to 3,692 (Table 1). Cultures of *Fusarium* spp. recovered from inoculated plants were identical to those used at inoculation. *F. oxysporum* and *F. graminearum* isolates were reisolated from root and crown, whereas *Fusarium* sp. nov. was reisolated from root, crown, and petiole of infected plants inoculated with *F. oxysporum*, *F. graminearum*, and *F. sp. nov.*, respectively.

Discussion

Shaner et al. (37) defined concepts of pathogenicity and virulence in several ways. In this study, *Fusarium* spp. inoculated at the eight-leaf growth stage which were able to induce disease symptoms (median disease severity ≥ 1) different from the noninoculated check plants (median disease severity = 0) were considered pathogenic. Virulence was considered the variation in the degree of disease severity caused by each *Fusarium* isolate. This study showed distinct variation in pathogenicity and virulence among three *Fusarium* spp. and among the isolates within each species found and collected from Minnesota sugar beet fields. Interestingly, isolates of a *Fusarium* sp. nov. showed higher frequency of pathogenicity and higher degree of virulence and, typically, induced disease symptoms earlier than the isolates of *F. oxysporum* and *F. graminearum* (Fig. 1). This would suggest that sugar beet fields with *Fusarium* sp. nov. will be more likely to have more severe disease than fields with *F. oxysporum* and *F. graminearum* under similar favorable environmental conditions. Moreover, *Fusarium* sp. nov. has been reported only from the Red River Valley of North Dakota and Minnesota (33). Regression analysis showed there was a relationship ($R^2 = 0.64$, $P < 0.01$; Fig. 2) between number of days required for symptom development and disease severity. In most instances, the more-virulent isolates of each species induced foliar symptoms earlier than less-virulent isolates (Table 1).

In all, 80% of the 98 isolates collected and identified from three *Fusarium* spp. in Minnesota in 2005 were pathogenic to sugar beet. The frequency of pathogenic isolates of *Fusarium* sp. nov. was higher (92%) than *F. oxysporum* (83%) and *F. graminearum* (57%). Associations of multiple *Fusarium* spp. on *Fusarium* yellows-infected sugar beet have been reported; however, the frequency of pathogenic isolates and degree of virulence among the isolates varied in the different studies. High frequency (82.5%) of pathogenic isolates of *F. oxysporum* was reported from Texas (21). In

Fig. 1. Frequency distribution of isolates from three *Fusarium* spp. according to their virulence on sugar beet in greenhouse assays. Severity classes are based on a 0-to-4 rating scale, where 0 = no symptoms. Classes are median ratings for five plants: 0 to 0.9 = nonpathogenic, median ratings 1 to 1.9 = less virulent, median ratings 2 to 2.9 = moderately virulent, and median ratings 3 to 4 = highly virulent.

Fig. 2. Relationship between disease severity and days required after inoculation for the first symptoms to appear in greenhouse assays with three different *Fusarium* spp. comprising 78 pathogenic isolates. Regression analysis showed that days required after inoculation for first symptoms to appear had a negative relationship ($R^2 = 0.64$; $P < 0.01$) with disease severity. Each data point is the average of ten observations.

contrast, Hanson and Hill (17) and Ruppel (33) found that 21 and 25% of their isolates, respectively, obtained from a different geographic area were pathogenic. The higher frequency of pathogenic isolates found in this study suggests that these isolates were probably more competitive and virulent. Each of the studies—Harveson and Rush (21,22), Hanson and Hill (17), Ruppel (34), and ours—used different *Fusarium* spp.-susceptible cultivars of sugar beet with different inherent levels of resistance to *Fusarium* yellows, which will also influence pathogenicity of the isolates (18). We do not know why *Fusarium* yellows complex, which was not considered a major problem for many years, suddenly began to infect numerous sugar beet fields in North Dakota and Minnesota starting around 2002. Around that time, Rhizomania, caused by *Beet necrotic yellow vein virus* and vectored by *Polymyxa betae* had become widespread and was starting to cause significant yield losses, and growers were adopting Rhizomania-resistant cultivars (19). It was observed that cultivar Van der Have 46177, a specialty cultivar approved because of good partial resistance to both Rhizomania and *Aphanomyces cochlioides*, which causes *Aphanomyces* root rot, was the most susceptible cultivar to *Fusarium* spp. in field and greenhouse studies (4,31). It was possible that some of the newer Rhizomania-resistant cultivars had less resistance to *Fusarium* spp.

Another possible reason for more *Fusarium* yellows complex in the Moorhead Factory District may be the presence of *Fusarium* sp. nov., which can be very virulent and occurred more frequently than *F. oxysporum*. Several researchers have reported new or different additional *Fusarium* spp. that cause diseases in other crops, including asparagus and chickpea (9,24). Similar to our study, Jimenez-Fernandez et al. (24) reported *F. redolens*, a new causal agent of *Fusarium* yellows in chickpea, which resulted in foliar symptoms similar to those of *F. oxysporum* f. sp. *ciceris*, previously considered as the principal causal agent of the disease on chickpea.

All the pathogenic isolates from the three *Fusarium* spp. showed similar foliar symptoms, including leaf yellowing (half- to full-leaf yellowing), interveinal yellowing, chlorosis, and necrosis of leaves, and most also induced vascular discoloration of the tap root and secondary roots. However, the more-virulent isolates generally resulted in more vascular discoloration than the less-virulent isolates. As such, *F. graminearum* isolates induced the lowest vascular discoloration and half-leaf yellowing. Because none of the roots had external symptoms, this will imply that the *Fusarium* disease present in Minnesota and North Dakota is distinctly different from the *Fusarium* root rot in Texas, where root tips are distinctly discolored and rotted (20). In some roots, cross sections showed decay of the entire central stele (0.5 to 1.0 cm in diameter) while most of the outer stele was healthy. Isolates that caused yellowing, chlorosis, and necrosis on foliar parts would adversely impact plant health and photosynthetic area. As such, infected sugar beet plants in fields will be expected to have reduced growth and yield potential (11).

In Minnesota and North Dakota, sugar beet is grown in rotation with wheat (spring, winter, and durum) barley, soybean, and corn (40), with predominantly wheat, barley, or corn being the preceding crop. *F. graminearum* is the primary *Fusarium* pathogen of wheat in Minnesota and North Dakota (29). Isolates of *F. graminearum* collected from wheat and sugar beet were genetically similar and belong to the same clade of *F. graminearum* complex (6) and were able to cause disease on both hosts alternatively (7). Although *F. graminearum* was shown to be pathogenic in this study, all isolates were less pathogenic, which suggests that it may not be a damaging pathogen of the sugar beet plant under the conditions evaluated. However, because *F. graminearum* causes dry rot on injured sugar beet root (12), it could pose problems to injured roots during the storage period.

The pathogen was reisolated from the root and crown of all inoculated seedlings with both pathogenic and nonpathogenic isolates. Interestingly, pathogenic isolates of *Fusarium* sp. nov. were also reisolated from the petiole, probably because it grew quickly and moved through the vascular system faster than other species. The

isolation of *Fusarium* spp. from the inner core of inoculated symptomless roots is consistent with previous studies (17,34). Reisolation of *Fusarium* spp. from symptomless roots suggests that the fungus can survive in plants without stimulating a pathogenic response.

Previously, severities of *Fusarium* yellows of sugar beet were evaluated up to 42 DAI for pathogenicity and virulence studies (16,17,34,41). Our preliminary study showed that some isolates initiated symptoms from 30 to 38 DAI. In this study, plants that developed yellowing symptoms of oldest leaves 30 to 38 DAI looked healthy later, when the initial infected leaves died. Isolates that caused initial yellowing symptoms to appear 25 and more DAI were all less virulent. Therefore, there would have been no differences in the number of days required to induce first symptoms or the disease severity of the isolates if the test was concluded 42 DAI. As such, future pathogenicity evaluations should be no longer than 42 DAI because the rankings of pathogenicity and virulence will not change.

In conclusion, isolates of three *Fusarium* spp. were characterized and evaluated for pathogenicity and virulence on a susceptible sugar beet host. *Fusarium* sp. nov. was the most commonly pathogenic, followed by *F. oxysporum* and *F. graminearum*. All of the *F. graminearum* isolates were less virulent and may not cause serious damage to sugar beet. Seed companies that develop *Fusarium* spp.-resistant cultivars for Minnesota and North Dakota should include both *F. oxysporum* and *Fusarium* sp. nov. when screening cultivars and conduct field studies in areas where both *F. oxysporum* and *Fusarium* sp. nov. are present.

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