Cross Pathogenicity and Vegetative Compatibility of *Fusarium oxysporum* Isolated from Sugar Beet

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Abstract

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Fusarium oxysporum f. sp. *betae* causes Fusarium yellows in sugar beet (*Beta vulgaris*). The *F. oxysporum* population from sugar beet can be highly variable in virulence and morphology and many isolates are nonpathogenic. Rapid and reliable methods to identify pathogenic isolates from nonpathogenic *F. oxysporum* generally are unavailable. Little is known about nonpathogenic isolates, including the role they may play in population diversity or virulence to sugar beet. Sugar beet is often grown in rotation with other crops, including dry edible bean (*Phaseolus vulgaris*) and onion (*Allium cepa*), with *F. oxysporum* able to cause disease on all three crops. Thirty-eight *F. oxysporum* isolates were collected from symptomatic sugar beet throughout the United States to investigate diversity of the *F. oxysporum* population and the influence of crop rotation on pathogenic variation. These isolates were characterized for pathogenicity to sugar beet, dry edible bean, and onion, as well as vegetative compatibility. Pathogenicity testing indicated that some *F. oxysporum* isolates from sugar beet may cause disease on onion and dry edible bean. Furthermore, vegetative compatibility testing supported previous reports that *F. oxysporum* f. sp. *betae* is polyphyletic and that pathogenic isolates cannot be differentiated from nonpathogenic *F. oxysporum* using vegetative compatibility.

Isolates of *Fusarium oxysporum* Schlechtend. are common soilborne fungi that typically cause wilting and root, tuber, or bulb rots in a wide range of host plant species (16). *F. oxysporum* isolates are categorized into formae speciales based on their ability to cause disease symptoms on specific hosts (4,7). *F. oxysporum* is a species complex of morphologically indistinguishable strains (5,31), with the assumption that isolates within a forma specialis (host specificity) are more similar genetically than to isolates with other host specificities (28). However, there are some instances where isolates within a forma specialis may be more related to nonpathogenic isolates or isolates that belong to different formae speciales (28,31,41). To date, there have been over 120 formae speciales described (15,35). Of interest for this work, *F. oxysporum* f. sp. *betae* infects sugar beet (*Beta vulgaris*), *F. oxysporum* f. sp. *cepae* infects onion (*Allium cepa*), and *F. oxysporum* f. sp. *phaseoli* infects dry edible bean (*Phaseolus vulgaris*).

Fusarium yellows of sugar beet, caused by *F. oxysporum* f. sp. *betae* (42,46,47), can cause significant reductions in root yield, sucrose percentage, and juice purity in affected plants (22,47). Fusarium yellows occurs throughout most sugar beet production areas of the United States (18,24,37,47,51). Symptoms of Fusarium yellows include interveinal yellowing, chlorosis, wilting and necrosis of the leaves, and a gray to brown or reddish-brown discoloration in the vascular tissue of the roots (19,22,42,47). *F. oxysporum* has also been associated with a second disease of sugar beet, Fusarium tip-rot (or Fusarium root rot), characterized by a severe tip rot and sometimes found in addition to the wilting and vascular discoloration associated with Fusarium yellows (25). Harveson and Rush (24,25) found that isolates that caused a Fusarium tip-rot were genetically and morphologically similar and, therefore, proposed that these isolates be classified as *F. oxysporum* f. sp. *radices-betae*. Currently, the most effective management strategy is through the use of resistant cultivars and crop rotations with nonhosts (22).

F. oxysporum isolated from sugar beet can be highly variable in growth, pigmentation, conidial production, and virulence (19,24,42). In addition to pathogenic strains of *F. oxysporum* f. sp. *betae,* many *F. oxysporum* strains isolated from symptomatic sugar beet are nonpathogenic in greenhouse pathogenicity testing (19,26,42,49). Classification of *F. oxysporum* f. sp. *betae* is made more difficult because many of these isolates can also infect other hosts such as spinach (*Spinacia oleraceae*) (3) as well as other weed species (33). Variability in the sugar beet *F. oxysporum* population is further enhanced when considering the wide geographic distribution of sugar beet production, diversity of initial fungal populations, and differing environmental influences (21,22,24,55).

Sugar beet production in eastern Colorado, eastern Montana, western Nebraska, and Wyoming, can occur in rotation with dry edible bean or, occasionally, onion (44,50). *F. oxysporum* can cause significant disease in both of these crops, with Fusarium wilt in dry edible bean caused by *F. oxysporum* f. sp. *phaseoli* (45) and Fusarium basal rot in onion caused by *F. oxysporum* f. sp. *cepae* (32). Because dry edible bean and onion are grown in rotation with sugar beet, each host could have a selective influence on the population of *F. oxysporum* in fields where these crops are rotated if those isolates are either cross-pathogenic or if sugar beet is acting as an asymptomatic host of *F. oxysporum* f. sp. *phaseoli* or *F. oxysporum* f. sp. *cepae* (17,23). Therefore, variations in the soil population structure of *F. oxysporum* will impact how resistance should be managed and deployed. Little is known about factors that determine pathogenicity or the phylogenetic relationships among *F. oxysporum* f. sp. *betae*, *F. oxysporum* f. sp. *cepae*, and *F. oxysporum* f. sp. *phaseoli* isolates (11,26,48). These formae speciales are believed to be distinct based on their genetic and pathogenic relationships; however, little is known about how crop rotation impacts the pathogenic populations from season to season.

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When isolates are collected from diseased sugar beet, they typically are inoculated only to known susceptible sugar beet controls to determine whether they are pathogenic and, therefore, assigned to the forma specialis *F. oxysporum* f. sp. *betae* (18,19,21,24, 26,42,51). Isolates unable to cause Fusarium yellows symptoms are classified as nonpathogenic *F. oxysporum* (16,19,26,42,49). However, these nonpathogenic isolates traditionally have not been inoculated to other hosts, particularly those that may be used in rotation with sugar beet (such as dry edible bean or onion), to determine whether they are pathogenic to other hosts. Sugar beet can be a symptomatic and asymptomatic host of pathogens affecting other crops (3,16,17,33) but its importance in maintaining pathogens in the cropping system is not well understood. In this case, it is possible that sugar beet is acting as an asymptomatic host of *F. oxysporum* isolates that are pathogenic to other crops found in the rotation (such as dry edible bean or onion). The reverse can also be said of isolates collected from other hosts which act as an inoculum source for Fusarium yellows in sugar beet.

One method used to differentiate *F. oxysporum* into formae speciales and further into races or strains uses vegetative compatibility groups (VCGs) (9,27,40). This method identifies isolates that have a high degree of genetic relatedness based on the ability of two isolates within a VCG to form heterokaryons with each other, while strains that are not closely related do not (29,40). Although isolates within a VCG are monophyletic (40), because *F. oxysporum* is a species complex, it is possible for a single forma specialis to consist of more than one clonal line (corresponding to multiple VCGs) and, therefore, that pathogenicity to a particular plant species can be polyphyletic (5,16,27,35,36). Vegetative compatibility grouping is controlled by a number of different loci termed "vegetative incompatibility (*vic*) loci" that are dispersed throughout the genome (29). A mutation at a single *vic* locus prevents the formation of a stable heterokaryon; thus, strains that are closely related normally occur in the same VCG (29). Even in cases where a complex relationship between race and VCG exists, the use of vegetative compatibility is useful in understanding the phenotypic diversity within a pathogen population and, perhaps, in differentiating pathogenic from nonpathogenic isolates (28,29).

Understanding pathogenic and phylogenetic relationships between populations of *F. oxysporum* that occur in crop rotation is important to understand their respective interactions with sugar beet, predict the potential for disease development, and identify potential sources of durable resistance in sugar beet. Because of the variability in pathogenicity of the *F. oxysporum* population isolated from sugar beet, this study examined the pathogenic relationships of isolates on sugar beet and two crops grown in the same crop rotation (onion and dry edible bean). Additionally, we attempted to further characterize the genetic relatedness of this population utilizing vegetative compatibility grouping.

Materials and Methods

Isolates. Fifty-one isolates of *F. oxysporum* were obtained from the collection located at the United States Department of Agriculture–Agricultural Research Service Sugar Beet Research Unit (SBRU), Ft. Collins, CO, or from researchers in known sugar beet production areas in the United States (Table 1). Each isolate was originally single spored or hyphal tipped when received, and stored on filter papers in the SBRU culture collection using protocols described by Leslie and Summerell (30). Each isolate was assigned a "test name" for identification purposes for the duration of the studies (Table 1). Prior to starting experiments, all isolates were confirmed to be *F. oxysporum* based on cultural and morphological characteristics, as described by Leslie and Summerell (30). Thirteen isolates had been previously characterized (20,24,48,52) and were used as positive controls for pathogenicity and VCG testing. These isolates included five isolates of *F. oxysporum* from Texas that represented VCGs previously described for *F. oxysporum* f. sp. *betae* (24), two isolates as positive controls for the sugar beet pathogenicity testing, three isolates previously described for *F. oxysporum* f. sp. *cepae* (48), and three isolates that had been described for identifying *F. oxysporum* f. sp. *phaseoli* (45) (Table 1). Thirty-eight isolates which were originally collected from symptomatic sugar beet were tested for pathogenicity and used for vegetative compatibility testing. Thirteen isolates were from Colorado, five from Idaho, eight from Michigan, six from Minnesota, and six from Wyoming (Table 1). Isolates were randomly assigned to one of two "sets" which were then used to inoculate each of the following hosts (sugar beet, dry edible bean, and onion) for pathogenicity to each crop. All isolates were maintained on potato dextrose agar (PDA; Becton, Dickinson and Company), as described by Leslie and Summerell (30). Only those isolates that were able to produce necessary nitrate-nonutilizing (*nit*) mutants, as described below, were included in vegetative compatibility testing (Table 2).

Sugar beet pathogenicity tests. For each isolate set, susceptible sugar beet germplasm 'FC716' (38) was planted and maintained in a greenhouse according to procedures previously described by Hanson et al. (20). Six weeks after planting (five- to six-leaf growth stage), plants were removed from pots and rinsed under running tap water to remove soil, and roots placed in a conidial spore suspension (10^5 conidia/ml) prepared as described by Hanson and Hill (19) for 8 min with intermittent agitation. Roots of control plants were placed in sterile distilled water. Two sugar beet isolates were included as positive controls for all sugar beet pathogenicity testing (F09-012/Fob220a and F09-013/F04-H7). Ten individual plants per isolate were inoculated and replanted into 10-cm "cone-tainers" (Steuwe and Sons, Inc.) containing premoistened pasteurized potting mix (Farfard number 2-SV; American Clay Works). Immediately after inoculation, plants were placed in a greenhouse in a randomized complete block design with two replications (five plants each) for 2 days at approximately 22°C to reduce transplant shock, after which temperatures were raised to 28°C with16 h of daylight. All sugar beet isolates were repeated three times. Plants were rated weekly for Fusarium yellows symptoms for 6 weeks after inoculation using a 0-to-5 rating scale (20). A rating of 0 = no disease; 1 = leaves wilted, small chlorotic areas on lower leaves, most of leaf green; 2 = leaves showing interveinal yellowing; 3 = leaves have small areas of necrosis or becoming necrotic and dying, less than half of the leaves affected; 4 = more than half of leaves dead, plant stunted, most living leaves showing symptoms; and 5 = plant death.

For assessing ability to cause Fusarium tip-rot symptoms in sugar beet, a second set of plants was inoculated in the same manner as described above (two replications of five plants each). At 4 weeks after inoculation, roots were pulled from cone-tainers, rinsed with water to remove soil, and inspected for visible lesions on the roots. Roots were rated on a 0-to-3 scale, where 0 = healthy with no visible lesions, 1 = lesions and necrosis confined to feeder roots, 2 = tap root lesions or clear tip rot symptoms, and 3 = root (and plant) totally dead. This experiment was also repeated three times.

Dry edible bean pathogenicity tests. For each isolate set, seed of a dry edible bean (pink-seeded 'Viva') known to be susceptible to all pathogenic isolates of *F. oxysporum* f. sp. *phaseoli* (12) were germinated in 15-cm-diameter pots containing vermiculite. Seedlings were grown in a greenhouse with day and night temperatures of 32 and 16°C, respectively, for 21 days or until the first trifoliolate leaf was fully emerged. For inoculum preparation, fungal isolates were transferred onto petri plates with half-strength PDA (½PDA) and cultured at 25°C for 2 weeks. The day prior to inoculation, conidia were prepared as described by Hanson and Hill (19) and stored at 4°C overnight. The following day, inoculum concentration was diluted to 10^6 conidia/ml for each isolate, and an agitated solution (by strong swirling) was used for subsequent inoculations. A clipped root-dip method of inoculation, as described by Pastor-Corrales and Abawi (39) and later modified by Salgado and Schwartz (43), was used. Ten plants per treatment were removed from original pots, soil was rinsed off with water, the distal one-third of the roots were cut off with sterile scissors, and the clipped roots were placed in the inoculum for 5 min. The inoculated plants were transplanted into new 15-cm-diameter pots

with sterilized potting mix (Metro-Mix200; The Scotts Company) with one plant per pot. Plants were placed into a greenhouse in a randomized complete block design with two replications (five plants each) and maintained at 22 to 25°C with a 13-h photoperiod. After 2 to 3 days, temperatures were increased to 32 and 16°C (day and night, respectively). Negative control plants were inoculated with sterile distilled water and positive controls (F09-057/FOP 58, F09-059/Fop1, and F09-060/Fop 30/FOP-CO) were inoculated with each set. Plants were watered every other day as necessary for survival of plants. At 21 days after inoculation, plants were scored using the 1-to-9 CIAT disease severity scale (8), where 1 = no disease symptoms and completely healthy, 3 = 10% of leaf surface showing disease symptoms, 5 = 25% of leaf surface showing disease symptoms and some whole plant stunting, 7 = disease symptoms on 50% of leaves and severely stunted, and 9 = plant death. The experiment was repeated twice.

Onion pathogenicity tests. Yellow bulb onions of an unknown cultivar were purchased from a grocery store the week of inoculation. Mature bulbs of all varieties of yellow onions are susceptible to the pathogenic control isolate of *F. oxysporum* f. sp. *cepae* (F09-053/Foc201d) (K. Otto and H. F. Schwartz, *unpublished*). The two outermost scales were removed from each onion and bulbs were surface disinfected by placing the entire onion in a 10% bleach solution for approximately 60 s, then allowed to air dry overnight under a laminar flow hood at room temperature. Isolates were transferred onto petri plates with ½PDA that had been modified by adding a total of 10 g of agar (Becton, Dickinson and Company) per liter so that the media had a harder consistency than normal.

Table 1. *Fusarium oxysporum* isolates for cross-pathogenicity testing and assignment to vegetative compatibility groups

Test name[y]	Isolate name	Donor[z]	Host isolated from	Location collected	Year collected
F09-001	F5	R. M. Harveson	Sugar beet	Texas	…
F09-002	F127	R. M. Harveson	Sugar beet	Texas	1993
F09-003	F156	R. M. Harveson	Sugar beet	Texas	1994
F09-004	F88	R. M. Harveson	Sugar beet	Texas	1993
F09-006	F120	R. M. Harveson	Sugar beet	Texas	1993
F09-012	FOB220a	H. F. Schwartz	Sugar beet	Iliff, Colorado	1998
F09-013	F04-H7	B. J. Jacobsen	Sugar beet	Montana	2004
F09-014	F06-104	SBRU	Sugar beet	Brush, Colorado	2006
F09-015	F49	SBRU	Sugar beet	Colorado	2001
F09-016	F02-57	SBRU	Sugar beet	Colorado	2002
F09-017	FOB216a	H. F. Schwartz	Sugar beet	Crook, Colorado	1998
F09-018	FOB216b	H. F. Schwartz	Sugar beet	Crook, Colorado	1998
F09-019	FOB216c	H. F. Schwartz	Sugar beet	Crook, Colorado	1998
F09-021	Fo220d	SBRU	Sugar beet	Iliff, Colorado	1998
F09-022	FOB257a	H. F. Schwartz	Sugar beet	Brush, Colorado	1998
F09-023	FOB257c	H. F. Schwartz	Sugar beet	Brush, Colorado	1998
F09-024	FOB266a	H. F. Schwartz	Sugar beet	Padroni, Colorado	1998
F09-025	F07-33	L. Hanson	Sugar beet	Eaton, Colorado	2007
F09-026	F07-36	L. Hanson	Sugar beet	Colorado	2007
F09-027	F28	SBRU	Sugar beet	Colorado	2001
F09-028	FOB22	SBRU	Sugar beet	Sabin, Minnesota	1998
F09-029	FOB25	SBRU	Sugar beet	Sabin, Minnesota	1998
F09-030	Fo17	C. Windels	Sugar beet	Crookston, Minnesota	2004
F09-031	F04-Fo28	C. Windels	Sugar beet	Crookston, Minnesota	2004
F09-032	F05-77	SBRU	Sugar beet	Moorehead, Minnesota	2005
F09-033	F05-157	SBRU	Sugar beet	Renville, Minnesota	2005
F09-034	FOB309	SBRU	Sugar beet	Laramie, Wyoming	1999
F09-035	F10-1	SBRU	Sugar beet	Goshen Co., Wyoming	1999
F09-036	F03-9	SBRU	Sugar beet	Worland, Wyoming	2003
F09-037	F04-30	SBRU	Sugar beet	Wyoming	2004
F09-038	F05-256	SBRU	Sugar beet	Laramie, Wyoming	2005
F09-039	F05-260	SBRU	Sugar beet	Laramie, Wyoming	2005
F09-040	F278	C. Strausbaugh	Sugar beet	Nampa, Idaho	2008
F09-041	F296	C. Strausbaugh	Sugar beet	Bruneau, Idaho	2008
F09-042	F299	C. Strausbaugh	Sugar beet	Nampa, Idaho	2008
F09-043	F597	C. Strausbaugh	Sugar beet	Nampa, Idaho	2008
F09-044	F598	C. Strausbaugh	Sugar beet	Jerome, Idaho	2008
F09-045	F07-52	Linda Hanson	Sugar beet	Gratiot Co., Michigan	2007
F09-046	F08-174	L. Hanson	Sugar beet	Saginaw Co., Michigan	2008
F09-047	F08-184	L. Hanson	Sugar beet	Saginaw Co., Michigan	2008
F09-048	F05-284	L. Hanson	Sugar beet	Michigan	2005
F09-049	F08-10	L. Hanson	Sugar beet	Michigan	2008
F09-050	F08-11	L. Hanson	Sugar beet	Michigan	2008
F09-051	F08-13	L. Hanson	Sugar beet	Michigan	2008
F09-052	F08-49	L. Hanson	Sugar beet	Michigan	2008
F09-053	FOC 201d	H. F. Schwartz	Onion	Weld, Colorado	1992
F09-055	#21	H. F. Schwartz	Onion	Montrose, Colorado	1999
F09-056	#23	H. F. Schwartz	Onion	Montrose, Colorado	1999
F09-057	FOP 58	H. F. Schwartz	Dry bean	Spain	…
F09-059	Fop 1/ATCC18131	H. F. Schwartz	Dry bean	South Carolina	…
F09-060	Fop 30/FOP-CO/ATCC90245	H. F. Schwartz	Dry bean	Sedgwick, Colorado	…

[y] First five isolates were previously reported by Harveson and Rush (24) and included as positive controls for sugar beet vegetative compatibility testing. Last six isolates are positive controls for onion and dry edible bean vegetative compatibility testing. Isolates in bold were positive controls for sugar beet, onion, and dry edible bean pathogenicity testing.

[z] Current institution of isolate donors: Dr. Robert M. Harveson, University of Nebraska, Scottsbluff 69361; Dr. Howard F. Schwartz, Colorado State University, Ft. Collins 80523; Dr. Barry J. Jacobsen, Montana State University, Bozeman 59717; United States Department of Agriculture–Agricultural Research Service (USDA-ARS) Sugar Beet Research Unit (SBRU), Fort Collins, CO 80526; Dr. Linda E. Hanson, USDA-ARS, East Lansing, MI 48824; Dr. Carol E. Windels, University of Minnesota, Crookston 56716; and Dr. Carl Strausbaugh, USDA-ARS, Kimberly, ID 83341.

Cultures were incubated at 25°C for 2 weeks. On the day of inoculation, a sterile 5-mm-diameter cork borer was used to remove four cores around the radius of each onion. A sterile 3-mm-diameter cork borer was used to create plugs from each isolate, which were placed in each of the four cores per onion using a flame-sterilized inoculation needle. Uninoculated agar cores from ½PDA petri plates were used as a negative control. The onions were placed into 1-gal. zip-lock plastic bags with a moist paper towel and incubated at 25°C. Three onions were inoculated per isolate, with each isolate being tested twice. At 14 days after inoculation, onions were scored on a 1-to-5 disease rating scale, where 1 = no obvious infection or development beyond the inoculum plug; 3 = some evidence of mycelial growth beyond the plug into the onion tissue, with slight discoloration or cellular breakdown; and 5 = abundant mycelial growth beyond the plug into the onion tissue, with discoloration and breakdown apparent.

Disease analysis. All pathogenicity experiments were performed using an augmented split-block design (14). Isolates were randomly assigned to one of two sets which represent the blocking for this experiment. Each inoculation set was used for two (dry

Table 2. Pathogenicity and vegetative compatibility groups (VCGs) of *Fusarium oxysporum* isolates from sugar beet[x]

Test name	Isolate name	Fusarium yellows of sugar beet	Fusarium tip-rot of sugar beet	Fusarium wilt of dry bean	Fusarium basal rot of onion	VCG[y]
F09-001	F5	NP (1.34 ± 0.40)	P (0.83 ± 0.20)	P (1.54 ± 0.10)	NP (1.06 ± 0.20)	Self incompatible (A)
F09-002	F127	NP (1.03 ± 0.40)	NP (0.18 ± 0.20)	NP (1.03 ± 0.14)	P (1.61 ± 0.20)	027-1 (A)
F09-003	F156	NP (0.19 ± 0.40)	NP (0.39 ± 0.20)	NP (1.03 ± 0.14)	NP (1.05 ± 0.20)	027-1 (A)/Self Incompatible (B)
F09-004	F88	NP (0.54 ± 0.40)	NP (0.41 ± 0.20)	NP (1.03 ± 0.14)	NP (1.30 ± 0.20)	Self incompatible (B)
F09-006	F120	NP (1.19 ± 0.40)	NP (0.18 ± 0.23)	NP (1.03 ± 0.14)	NP (1.05 ± 0.20)	027-5 (A)
F09-012	FOB220a	PC (3.55 ± 0.90)	PC (0.97 ± 0.36)	NP (1.03 ± 0.14)	NP (1.06 ± 0.20)	VCG 8 (027-8) (B)
F09-013	F04-H7	PC (2.89 ± 0.90)	PC (0.93 ± 0.36)	NP (1.03 ± 0.14)	NP (1.06 ± 0.20)	Self incompatible (B)
F09-014	F06-104	NP (0.98 ± 0.40)	NP (0.19 ± 0.20)	NP (1.03 ± 0.14)	NP (1.05 ± 0.20)	Single member VCG (B)
F09-015	F49	NP (1.28 ± 0.40)	NP (0.52 ± 0.20)	NP (1.03 ± 0.14)	NP (1.12 ± 0.20)	Single member VCG (B)
F09-016	F02-57	NP (1.00 ± 0.40)	NP (0.29 ± 0.20)	NP (1.33 ± 0.14)	NP (1.05 ± 0.20)	Self incompatible (B)
F09-017	FOB216a	P (4.23 ± 0.40)	NP (0.43 ± 0.20)	P (2.02 ± 0.14)	NP (1.06 ± 0.20)	VCG 8 (027-8) (B)
F09-018	FOB216b	NP (1.11 ± 0.40)	NP (0.50 ± 0.20)	NP (1.03 ± 0.14)	NP (1.05 ± 0.20)	VCG 10 (042-4)[BC] and VCG 14 (042-1) (B)
F09-019	FOB216c	NP (1.74 ± 0.40)	NP (0.69 ± 0.20)	NP (1.03 ± 0.14)	P (2.23 ± 0.20)	Single member VCG (B)
F09-021	Fo220d	NP (0.53 ± 0.40)	NP (0.14 ± 0.20)	NP (1.03 ± 0.14)	NP (1.05 ± 0.20)	Not tested (B)
F09-022	FOB257a	NP (0.93 ± 0.40)	NP (0.47 ± 0.20)	NP (1.03 ± 0.14)	NP (1.18 ± 0.20)	Single member VCG (B)
F09-023	FOB257c	P (3.01 ± 0.40)	P (1.45 ± 0.20)	NP (1.03 ± 0.14)	NP (1.05 ± 0.20)	VCG 12 (027-12) (B)
F09-024	FOB266a	P (2.87 ± 0.40)	P (1.13 ± 0.20)	NP (1.03 ± 0.14	NP (1.05 ± 0.20)	VCG 8 (027-8) (B)
F09-025	F07-33	NP (1.18 ± 0.46)	NP (0.29 ± 0.23)	NP (1.03 ± 0.14)	NP (1.05 ± 0.20)	VCG 11 (027-11) (B)
F09-026	F07-36	NP (1.05 ± 0.40)	NP (0.61 ± 0.20)	NP (1.03 ± 0.14)	NP (1.06 ± 0.20)	Not tested (B)
F09-027	F28	NP (0.56 ± 0.40)	NP (0.14 ± 0.20)	NP (1.03 ± 0.14)	P (1.77 ± 0.20)	Not tested (B)
F09-028	FOB22	NP (0.96 ± 0.40)	NP (0.32 ± 0.20)	NP (1.03 ± 0.14)	NP (1.35 ± 0.20)	Self incompatible (B)
F09-029	FOB25	NP (1.01 ± 0.40)	NP (0.25 ± 0.20)	NP (1.03 ± 0.14)	NP (1.06 ± 0.20)	Not tested (B)
F09-030	Fo17	NP (0.45 ± 0.40)	NP (0.25 ± 0.20)	NP (1.03 ± 0.14)	NP (1.12 ± 0.20)	Single member VCG (B)
F09-031	F04-Fo28	P (2.40 ± 0.40)	P (1.07 ± 0.20)	NP (1.03 ± 0.14)	NP (1.05 ± 0.20)	VCG 12 (027-12) (B)
F09-032	F05-77	NP (0.60 ± 0.40)	NP (0.25 ± 0.20)	NP (1.03 ± 0.14)	NP (1.06 ± 0.20)	Not tested (B)
F09-033	F05-157	NP (0.79 ± 0.40)	NP (0.74 ± 0.20)	NP (1.07 ± 0.14)	P (2.53 ± 0.20)	VCG 13 (042-) (B)
F09-034	FOB309	P (2.76 ± 0.40)	P (0.92 ± 0.20)	NP (1.03 ± 0.14)	NP (1.05 ± 0.20)	Self incompatible (B)
F09-035	F10-1	NP (0.77 ± 0.40)	NP (0.36 ± 0.20)	NP (1.03 ± 0.14)	NP (1.06 ± 0.20)	Not tested (B)
F09-036	F03-9	NP (0.66 ± 0.46)	NP (0.25 ± 0.27)	NP (1.03 ± 0.14)	NP (1.06 ± 0.20)	Single member VCG (B)
F09-037	F04-30	NP (0.54 ± 0.40)	NP (0.25 ± 0.20)	NP (1.10 ± 0.14)	NP (1.06 ± 0.20)	Single member VCG (B)
F09-038	F05-256	NP (0.99 ± 0.40)	NP (0.14 ± 0.20)	NP (1.03 ± 0.14)	NP (1.15 ± 0.20)	Not tested (B)
F09-039	F05-260	NP (0.60 ± 0.40)	NP (0.47 ± 0.20)	NP (1.03 ± 0.16)	NP (1.18 ± 0.20)	Single member VCG (B)
F09-040	F278	NP (0.62 ± 0.40)	NP (0.47 ± 0.20)	NP (1.03 ± 0.14)	NP (1.12 ± 0.20)	Single member VCG (B)
F09-041	F296	NP (1.24 ± 0.40)	NP (0.25 ± 0.20)	NP (1.03 ± 0.14)	NP (1.12 ± 0.20)	Single member VCG (B)
F09-042	F299	NP (1.40 ± 0.40)	NP (0.47 ± 0.20)	NP (1.11 ± 0.14)	NP (1.05 ± 0.20)	Self incompatible (B)
F09-043	F597	NP (0.62 ± 0.40)	NP (0.18 ± 0.20)	NP (1.03 ± 0.14)	NP (1.35 ± 0.20)	Not tested (B)
F09-044	F598	NP (0.87 ± 0.40)	NP (0.14 ± 0.20)	NP (1.03 ± 0.14)	NP (1.12 ± 0.20)	VCG 13 (042-) (B)
F09-045	F07-52	NP (1.24 ± 0.40)	NP (0.41 ± 0.20)	NP (1.07 ± 0.14)	NP (1.05 ± 0.20)	Not tested (B)
F09-046	F08-174	NP (0.67 ± 0.40)	NP (0.25 ± 0.20)	NP (1.07 ± 0.14)	NP (1.05 ± 0.20)	VCG 9 (027-9) (B)
F09-047	F08-184	NP (0.59 ± 0.40)	NP (0.45 ± 0.20)	NP (1.03 ± 0.14)	NP (1.24 ± 0.20)	VCG 9 (027-9) (B)
F09-048	F05-284	NP (0.79 ± 0.40)	NP (0.32 ± 0.20)	NP (1.03 ± 0.14)	NP (1.11 ± 0.20)	VCG 10 (042-4)[BC]
F09-049	F08-10	NP (0.83 ± 0.40)	NP (0.14 ± 0.20)	NP (1.03 ± 0.14)	NP (1.05 ± 0.20)	Self incompatible (B)
F09-050	F08-11	NP (0.86 ± 0.40)	NP (0.34 ± 0.20)	NP (1.03 ± 0.14)	NP (1.05 ± 0.20)	Not tested (B)
F09-051	F08-13	NP (1.09 ± 0.40)	NP (0.52 ± 0.20)	NP (1.11 ± 0.14)	NP (1.05 ± 0.20)	VCG 11 (027-11) (B)
F09-052	F08-49	NP (0.83 ± 0.40)	NP (0.72 ± 0.20)	NP (1.07 ± 0.14)	NP (1.06 ± 0.20)	Not tested (B)
F09-053	FOC 201d	NP (0.84 ± 0.40)	NP (0.23 ± 0.20)	NP (1.03 ± 0.14)	PC (5.00 ± 0.39)	VCG 14 (042-1)[BC]
F09-055	#21	NP (0.67 ± 0.46)	NP (0.47 ± 0.23)	NP (1.03 ± 0.14)	NP (1.06 ± 0.20)	VCG 10 (042-4)[BC]
F09-056	#23	NP (0.78 ± 0.46)	NP (0.32 ± 0.23)	NP (1.03 ± 0.14)	NP (1.05 ± 0.20)	Onion VCG #5 (C)
F09-057	FOP 58	NP (1.33 ± 0.40)	NP (0.14 ± 0.20)	PC (9.00 ± 0.25)	NP (1.52 ± 0.20)	Not tested (B)
F09-059	Fop 1/ATCC18131	NP (0.81 ± 0.46)	NP (0.32 ± 0.23)	PC (9.00 ± 0.25)	NP (1.58 ± 0.20)	016-1 (D)
F09-060	Fop 30/FOP-CO/ATCC90245	NP (0.97 ± 0.46)	NP (0.32 ± 0.23)	PC (9.00 ± 0.25)	NP (1.41 ± 0.20)	016- (D)
WA[z]	…	NC (0.81 ± 0.90)	NC (0.03 ± 0.36)	NC (1.00 ± 0.25)	NC (1.10 ± 0.39)	Not tested (B)

[x] Pathogenicity data: PC = positive control isolate; NC = negative control, dipped in water or agar plug; NP = nonpathogen; WP = weak pathogen; P = pathogen. Numbers in parentheses indicate mean disease severity rating (± standard error of the mean). All isolates considered to be pathogens were significant at $P < 0.05$ based on comparisons of the adjusted best linear unbiased estimates (Blups) with the estimated means for the positive and negative controls for each host.

[y] Letters in parentheses indicate source of VCG associations: A, Harveson and Rush (24); B, Swift et al. (48); and C, Woo et al. (52). VCG designations without a source are from this study.

[z] Water or agar plug.

edible bean and onion) or three (sugar beet) inoculation dates (experiments). At each inoculation date, two replicates were performed where each isolate was randomly inoculated to a block of plants (five plants for sugar beet, four plants for dry edible bean, and three bulbs for onion) per replicate. Statistical analyses were conducted using SAS Proc Glimmix (version 9.2; SAS Institute) and the best linear unbiased estimates (Blups) were compared with the respective negative and positive controls for each host. Isolates considered to be pathogenic to each host had Blups that were significantly different from the controls ($P < 0.05$). Mean values are followed by standard error of the mean.

VCG testing. All isolates were transferred to PDA with varying amounts of potassium chlorate (1.5 to 6%) in order to recover *nit* mutant isolates for vegetative compatibility testing (30). Mutants were then transferred to complete media (30) containing nitrate as the sole nitrogen source and incubated for 3 to 4 days until thin cultures had grown. Putative *nit* mutants (cultures that grow thinly) were transferred to four phenotyping media (NO_3, NH_4, NO_2, and HX) to determine which enzyme in the nitrate reductase pathway was affected in the *nit* mutants (9,24). This process was continued until a *nit1* and a *nitM* mutant was identified for each isolate used for the study. For isolates in which the recovery of *nitM* mutants was difficult, isolates were plated onto 5 to 6.5% potassium chlorate media which had proline in replacement of the L-asparagine, as recommended by Leslie and Summerell (30). Isolates were grown on complete media (30) slants for 7 to 10 days at 25°C in the dark. Approximately 1 ml of sterile 2% Tween 60 solution was added to each slant to make a spore suspension. For each isolate pairing, a Pasteur pipette was used to add 1 drop of the spore suspension from each respective isolate to opposite sides of a single well in a 24-well plate containing minimal media and allowed to dry. Inoculated 24-well plates were incubated for 7 days at 25°C in the dark and scored for heterokaryon formation (dense aerial growth at the anastomosis zone) (9,30). Only VCGs that contained two or more isolates were assigned to a VCG (24,48). VCG number assignments were made according to the Puhalla numbering system (40) and previous reported work by Harveson and Rush (24) and Swift et al. (48). All isolates were tested twice. Isolates that had conflicting results between replications were paired with each other a third time.

Results

Pathogenicity tests. Not all isolates originally collected on symptomatic sugar beet caused Fusarium yellows symptoms when inoculated on susceptible sugar beet. Only 5 of the 38 isolates (approximately 13%) differed from the water control plants but were similar to the positive control isolates (F09-012 and F09-013, *P* values of <0.0001 to 0.0018) and were classified as pathogenic (Table 2). Three of the pathogenic isolates were originally collected from Colorado (F09-017, F09-023, and F09-024), one from Minnesota (F09-031), and one from Wyoming (F09-034) (Table 1). Four of the five isolates which were pathogenic for Fusarium yellows also caused tip-rot symptoms on sugar beet, with only F09-017 not causing both symptoms (Table 2; *P* values of <0.0001 to 0.0427). A sixth isolate (F09-001) also caused a tip-rot symptom and was originally described by Harveson and Rush (24); however, this isolate did not cause Fusarium yellows (Table 2). Of the four sugar beet isolates that were pathogenic as Fusarium yellows and Fusarium tip-rot, only one isolate (F09-023) had a clear tip-rot symptom on the tap root, whereas all other isolates caused a general necrosis on the feeder roots rather than a clear tip rot.

All *F. oxysporum* f. sp. *phaseoli* isolates (used for positive controls) were pathogenic on dry bean, as expected. Two of the sugar beet isolates caused disease symptoms on dry bean (Table 2). These two isolates included one of the isolates originally published by Harveson and Rush (24) (F09-001, $P = 0.001$), as well as one of the pathogenic *F. oxysporum* from sugar beet (F09-017, $P < 0.0001$).

All isolates were also tested for ability to cause Fusarium basal rot of onion, with pathogenicity assigned if they were significantly different from the negative control (sterile agar plug). Three isolates originally collected from sugar beet were pathogenic on onion (F09-019, F09-027, and F09-033; values of $P < 0.0001$ to $P = 0.0049$; Table 2). A fourth sugar beet isolate that had been previously described by Harveson and Rush (24) also was pathogenic to onion (Table 2; $P = 0.0379$). Although this isolate (F09-002) previously caused a Fusarium tip-rot symptom on sugar beet (24), in our study, this isolate was nonpathogenic on sugar beet (Table 2).

Vegetative compatibility tests. The mutants *nit1* and *nitM,* needed to assess VCGs (Table 2), were recovered from 39 of the 51 isolates. Nine of these isolates (F09-001, F09-003, F09-004, F09-013, F09-016, F09-028, F09-034, F09-042, and F09-049) were self incompatible and, thus, were not included in pairing studies. A 10th isolate, F09-038, reverted back to a wild type during pairing experiments and was excluded from the VCG analysis. Therefore, 29 isolates remained for pairing into VCGs. For positive controls, two isolates originally described by Harveson and Rush (24) were used to represent sugar beet—VCG 1 (027-1; F09-002) and VCG 5 (027-5; F09-006)—and each was capable of producing both *nit* mutants needed to confirm VCGs from their work; however, neither isolate paired with any other isolate in this study. Additional isolates representing published sugar beet VCGs obtained from Dr. R. Harveson (University of Nebraska, Scottsbluff) either could no longer be identified as *F. oxysporum* or were self incompatible in our testing (F09-003 and F09-004). Therefore, we did not have controls of all previously reported sugar beet VCGs. Because of this, VCG numbering was assigned assuming new VCGs and consistent with previous numbering protocols (24,27).

Seven new VCGs were found, as compared with those previously described by Harveson and Rush (24), each containing at least two or more members. These new VCGs were numbered VCG 8 (027-8), VCG 9 (027-9), VCG 10 (042-4), VCG 11 (027-11), VCG 12 (027-12), VCG 13 (042-), and VCG 14 (042-1) (Table 2). The remaining 10 isolates paired successfully with themselves but did not pair with other isolates included in the study (Table 2). Therefore, those isolates were classified as single-member VCGs, as described previously by Harveson and Rush (24). All isolates that grouped into VCG 8 (027-8) were originally collected from Colorado and were pathogenic to sugar beet. Three of these isolates (F09-12, F09-017, and F09-024) were collected from the north-central production region along the Colorado Front Range. VCG 9 (027-9) contained two isolates that were collected from Michigan, and both were nonpathogenic to sugar beet. VCG 10 had one of the *F. oxysporum* f. sp. *cepae* isolates that was used as a positive control (F09-055) (Table 2). This isolate had been previously assigned an onion VCG (042-4) (48) and was collected from Montrose, CO. The two isolates that were assigned to VCG 10 (F09-018 and F09-048) were both nonpathogenic to any of the host species tested and were collected from sugar beet in Colorado (1998) and Michigan (2005), respectively. Because this VCG had previously been described for onion (48) and the new isolate was not considered to be pathogenic to sugar beet, we have assigned the onion VCG (042-4) to this group. Interestingly, F09-018 also paired with another *F. oxysporum* f. sp. *cepae*-positive control isolate that represented onion VCG 042-1 (F09-053). Therefore, we assigned this group as an onion VCG 14 (042-1). VCG 11 (027-11) and VCG 12 (027-12) both contained two isolates each, with VCG 12 containing isolates pathogenic to sugar beet while VCG 11 had nonpathogenic isolates. These isolates were collected from different production regions (Colorado and Michigan). VCG 13 contained one isolate that was not pathogenic to sugar beet but was pathogenic to onion. This isolate (F09-033) did not pair with either of the representative onion isolates but did pair with an *F. oxysporum* isolate that was nonpathogenic on the three crops tested. At this time, we assigned an onion VCG to this group but did not number it because additional testing with an onion population would clarify which group this VCG belongs to with the onion numbering system (Table 2).

Discussion

Isolates of *F. oxysporum* are categorized into formae speciales according to their ability to cause diseases on specific hosts (4,7).

In this study, 38 isolates of *F. oxysporum* collected from symptomatic sugar beet were tested with three potential hosts to identify their putative forma specialis and determine whether they were pathogenic on sugar beet, dry bean, or onion. We found that only 13% (5 of 38) of these isolates were pathogenic to sugar beet and, therefore, were classified as *F. oxysporum* f. sp. *betae*. Ruppel (42) has previously reported that 40% of *F. oxysporum* that were isolated from symptomatic sugar beet were "pathogens" of the host. Hanson and Hill (19) reported a slightly lower rate of recoverable pathogenic *F. oxysporum* isolates collected from symptomatic sugar beet over the entire United States production region. Our rate of recovered pathogenic isolates was lower than these previous reports; however, many *F. oxysporum* f. sp. *betae* isolates with moderate or low virulence to sugar beet have been reported to give variable disease severity ratings (19,26), particularly when testing the same isolates in multiple locations, where there may be environmental differences in growing and testing conditions (19,26,32,34,47). Contributing to the high number of nonpathogenic isolates identified in this study, some isolates appeared to have a weakly pathogenic reaction when compared with the negative control. It is possible that these isolates could be considered as having low virulence to each of the hosts in question; however, we were not able to statistically demonstrate this in our studies. Identification of positive controls that demonstrate the range of virulence to each host (i.e., low, moderate, and high virulence) to be included in future pathogenicity testing could potentially resolve these ambiguous pathogen designations.

Harveson and Rush (24) had previously described sugar beet isolates that caused a tip-rot symptom. They suggested that these isolates were a separate forma specialis of *F. oxysporum* and should be classified as *F. oxysporum* f. sp. *radicis-betae* (24). However, in our study, we tested a few of these isolates and found that none were pathogenic to sugar beet for Fusarium yellows and only one was weakly virulent for Fusarium tip-rot. It is not known why the isolates from Harveson and Rush (24) were no longer pathogenic for Fusarium tip-rot, as previously described. We propose two possibilities. First, Harveson and Rush (24) utilized a different methodology of inoculation for identifying tip-rot symptoms, where the inoculum was poured directly over the crown. In this study, we utilized a root-dip assay which may have influenced symptom expression. Second, there were some discrepancies in both morphology and virulence for some of the isolates we received that were meant to represent the original isolates or VCGs reported by Harveson and Rush (24). Because of this, any isolate that had a difference in morphology from its original report was not used for this study, and any differences in virulence or vegetative compatibility grouping were indicated in this study. In addition to the difference in inoculation method, it is possible that the way the isolates were stored or maintained since 1997 may have somehow impacted these particular isolates (R. Harveson, *personal communication*). Both possibilities for our findings should be investigated further before using these isolates in future studies. Additionally, because of these issues, additional studies to standardize environmental and testing factors which could influence *F. oxysporum* f. sp. *betae* pathogenicity and virulence for both Fusarium yellows and Fusarium tip-rot development should be undertaken in order to better characterize what are true pathogens of sugar beet. We found that symptoms associated with tip-rot disease of sugar beet were not independent from symptoms of Fusarium yellows. In our study, isolates able to cause Fusarium yellow symptoms on sugar beet also produced some tip-rot necrosis on the roots. Additionally, the more virulent the isolate was in regards to Fusarium yellows development, the more severe the tip-rot symptoms were.

It has been previously established that sugar beet can harbor pathogens to other hosts (3,16,17,33). A small number of sugar beet isolates found in sugar beet that expressed symptoms while not pathogenic to sugar beet, were pathogenic to other plant species that can be found in rotation with sugar beet (i.e., dry edible bean and onion) (3,11,33). We predict that it is possible for *F. oxysporum* f. sp. *cepae* isolates to survive on sugar beet, potentially providing an inoculum load for the following onion crop. We have found no published work where isolates of *F. oxysporum* f. sp. *cepae* have been cross-inoculated to sugar beet. A better understanding of how much onion contributes to maintaining pathogen load in rotations with onion and sugar beet are needed and require further investigation.

The vegetative compatibility testing studies of Harveson and Rush (24), combined with our results, indicate that the *F. oxysporum* population from sugar beet is complex, supporting previous molecular work of others (11,26). Most of the isolates we tested were self compatible with themselves but did not pair with any other isolate in the test (single-member VCGs). Other researchers have reported a similarly large number of single-member VCGs for some formae speciales of *F. oxysporum* (1,6,13). They predicted that lack of heterokaryon formation among different isolates could result from mutation at one or more of the *vic* loci which prevents the formation of heterokaryons among classes of *nit* mutants (10,29). Of the seven additional VCGs that formed, typically, both pathogenic and nonpathogenic isolates were identified as members and there was no general trend that isolates were grouped based on the production region where they were originally collected (i.e., state). Two VCGs (027-8 and 027-9) contained only pathogenic isolates, whereas other VCGs had a mixture of pathogens and nonpathogens, and three VCGs appear to belong to onion pathogens. However, more isolates need to be included in a broader VCG study prior to making conclusions that "pathogenic" VCGs can be described in the *F. oxysporum* f. sp. *betae* population. Our work supports the work of Harveson and Rush (24), who found that isolates collected in Texas were generally more closely related to each other than to isolates from other regions. Our study also supports previous information that suggests that *F. oxysporum* populations from sugar beet are polyphyletic. Hill et al. (26), utilizing sequencing of conserved gene regions, demonstrated that *F. oxysporum* isolates from sugar beet were grouped loosely into multiple clades generally based on production region. However, due to the low numbers of isolates in each VCG, a more complete survey using additional isolates from each geographic region needs to be done before we can support any correlation to production region as well as to resolve the number of VCGs that represent the population.

Combining cross-pathogenicity data with vegetative compatibility testing, several sugar beet isolates were found to be pathogenic to onion (or grouped into onion VCGs). One isolate (F09-019) was collected from the same sugar beet root as two other isolates in the study (F09-017 and F09-018), with F09-017 isolated from the crown, F09-018 from mid-root, and F09-019 from the tip. F09-017 was pathogenic to sugar beet but not to onion. F09-018, although not pathogenic to either sugar beet or onion, fell into two previously described onion VCGs (042-4). More work needs to be done in determining the relationship of these three isolates, particularly because they may potentially represent multiple VCGs obtained from the same sugar beet. Also, one isolate (F09-018) was found to form a heterokaryon with two onion VCG groups (VCG 10 and VCG 14). Normally isolates within the same VCG are clonally related, although exceptions to this rule have been previously reported based on a phylogenetic study utilizing sequencing from the internal transcribed spacer-5.8S rDNA region (2). No information was reported regarding field rotation history of these particular isolates. The relationship of these isolates, the identification of an overlap in VCG relationships with onion, and the number of isolates that were found to be pathogenic to onion, in general, indicate that a more detailed survey of *F. oxysporum* from fields with a known sugar beet–onion rotation would be useful to determine whether there is a closer relationship between these two formae speciales than previously believed.

Previous phylogenetic analysis, done by Hill et al. (26) and Webb et al. (49), has reported that pathogenicity to sugar beet is polyphyletic and that the *F. oxysporum* population from the United States sugar beet production regions can be roughly broken out

into three clades, very loosely based on pathogenicity. Because of the complex nature of VCG results that we found, molecular phylogenetic characterization of the *F. oxysporum* population from sugar beet will be more informative to researchers and producers in the future, except perhaps when looking at isolates that may be cross pathogenic to other crops (such as onion).

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