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EVALUATING INOCULATION METHODS TO INFECT SUGAR BEET WITH *FUSARIUM OXYSPORUM* F. SP. *BETAE* AND *F. SECORUM*

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Abstract: Minnesota and North Dakota combined contain 55% of the sugar beet production area in the USA, contributing to 49% of the nation's sugar beet production in 2018. Fusarium diseases caused by *Fusarium oxysporum* f. sp. *betae* and *F. secorum* on sugar beet can cause significant reduction in both root yield and sucrose concentration and purity. The objective of this research was to identify an alternative artificial inoculation method to induce Fusarium diseases on sugar beet leaves and roots caused by both *Fusarium* species in greenhouse conditions to better aid in research efforts. We tested four inoculation methods, including barley to seed, barley to root, drenching, and cutting and compared them with the conventional root-dipping inoculation method. The inoculation method of placing *Fusarium* colonized barley seeds close to sugar beet seeds (barley to seed) caused similar levels of symptom severities both on leaves and roots as the root-dipping method. As the traditional root dipping method involves a

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laborious transplant process, use of infected barley seed as inoculum may serve as an alternative method in the evaluation of host resistance and pathogen virulence among Fusarium diseases by *Fusarium* spp. on sugar beet at the seed/seedling stage.

Keywords: Sugar beet, *F. oxysporum* f. sp. *betae*, *Fusarium secorum*, Root-dipping, and *Fusarium*-colonized barley seeds.

1. Introduction

Sugar beet (*Beta vulgaris* L.) is a major source of global sucrose production, especially in temperate regions (FAO, 2009). The United States was the fourth largest sugar beet producer in the world in 2017 (FAO, 2017). In 2018, Minnesota and North Dakota accounted for 55% of thesugar beet growing area and contributed 49% of the total sugar beet production in the USA (USDA-ERS, 2019). Diseases caused by *Fusarium* spp. on sugar beet can reduce root yield and extractable sucrose (Hanson and Jacobsen, 2009).

In the Red River Valley of North Dakota and Minnesota, the *Fusarium* spp. *F. oxysporum* f. sp. *betae* (D. Stewart) W.C. Snyder and H.N. Hansen and *F. secorum* are the pathogens most consistently associated with Fusarium diseases on sugar beet (Khan et al., 2009). Fusarium yellows caused by *F. oxysporum* f. sp. *betae* was first reported in the Red River Valley in 2002 (Windels et al., 2005). The disease symptoms are a characteristic interveinal chlorosis, internal taproot vascular-discoloration without external appearance, and canopy wilt. In 2005, a new disease Fusarium yellowing decline, caused by *F. secorum* was first reported by Rivera et al. (2008) in Minnesota (Secor et al., 2014). Unlike *F. oxysporum* f. sp. *betae*, only *F. secorum* causes seedling death, yellowing during early growing season, and petiole vascular discoloration (Burlakoti, 2012).

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Effective artificial inoculation methods are necessary for the identification of sources of host resistance, host-pathogen interactions, and studies on disease control strategies (Das and Patil, 2015). The root-dipping inoculation method has been the standard method for evaluating *F. oxysporum* infection, which affects several plant species including chickpea, tomato, cotton, and cucumber (Dowd et al., 2004; Maitlo et al., 2016; Rowe, 1980; Vakalounakis, 1996). Root-dipping method has also been used to evaluate *F. oxysporum* f. sp. *betae* on sugar beet (Hanson, 2006). This same inoculation method has been used to study the effect of *F. secorum* on sugar beet (Burlakoti, 2007; Rivera et al., 2008). The root-dipping inoculation method involves damaging theroots, allowing the pathogen to invade through wounds, avoiding a natural barrier at the epidermis (Eynck et al., 2009). Alternative inoculation methods which do not result in artificial wounding of the root like the standard root-dipping method would be of value as they better simulate natural conditions during pathogen attempts at establishment. In this work, we tested four alternative inoculation methods to identify a more effective inoculation method for Fusarium disease evaluations.

2. Materials and methods

2.1 Fungal isolates

Known pathogenic isolates *F. oxysporum* f. sp. *betae* F-19, isolated from Salem, Oregon in 2001, and provided by the USDA-ARS Sugarbeet Research Unit, Fort Collins, Colorado (CO), and *F. secorum* 784-12-4, isolated from Sabin, Minnesota in 2007, provided by Dr. G. A. Secor, North Dakota State University, Fargo, North Dakota (ND) were used for this study.

2.2 Inoculum preparation

Liquid cultures were prepared using CarboxyMethylCellulose (CMC) medium. One liter of CMC medium contains 15 g of carboxymethylcellulose sodium salt (Sigma-Aldrich, USA), 1

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g of ammonium nitrate (ACS reagent, ≥98%; Sigma-Aldrich, USA), 1 g of potassium phosphate monobasic (Sigma-Aldrich, USA), 0.5 g of magnesium sulfate heptahydrate (ACS reagent, ≥98%; Sigma-Aldrich, USA), and 1 g of yeast extract (Sigma-Aldrich, USA). All chemicals were dissolved in one liter distilled water and autoclaved at 170 kPa and 120°C for 20 min. Fungal cultures were prepared by transferring hyphae from a long term storage vial into 100 × 15 mm petri dishes (Falcon, USA) containing full strength potato dextrose agar (PDA)(Sigma-Aldrich, USA), and incubating them under fluorescent light at room temperature (24°C) for one week. Erlenmeyer flasks containing 200 ml of CMC medium was inoculated with 20 pieces of 5 mm^2 plugs containing actively growing hyphae. The inoculated CMC medium was placed in a rotary shaker (Thermo Scientific MaxQ Shakers, USA), and incubated at 210 rpm under soft white fluorescent light at 25°C. After 7 days, the CMC medium was passed through 2-layers of miracloth (Calbiochem, EMD Millipore Corporation, Billerica, USA) to collect spores. A hemocytometer (Propper Manufacturing Co., Inc., USA) was used to estimate the concentration. The spore suspension was adjusted to 5×10^4 spores/ml with distilled water and used immediately.

Barley seeds (non-treated) were used as a solid substrate. *Fusarium*-infested barley inoculum were produced following the same method used for producing *Rhizoctonia solani*-infested barley grains (Kirk et al., 2008; Noor and Khan, 2014). Mixtures of 4.8 g potato dextrose broth (PDB; Sigma-Aldrich, USA), 200 ml barley, and 120 ml distilled water (5:3barley:distilled water v/v ratio) were placed into 500-ml flasks (Pyrex, USA) and autoclaved at 170 kPa and 120°C for 30 min, then left to cool to room temperature overnight. The initial inoculum was grown on PDA as described above, cut into 3 mm^2 plugs and transferred into autoclaved flasks containing barley. One flask of barley was inoculated with plugs from one petri dish. Inoculated flasks were sealed, mixed every two days by hand-shaking, incubated at room

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temperature for two weeks and then air dried under a laminar flow hood for 2-days. The air-dried barley grains were stored at 4°C until used. Colony forming units (CFU) were calculated for each isolate by grinding 50 grains in 100 ml autoclaved distilled water for 5 min using a blender. Three serial 1/10 dilutions were prepared and a total volume of 100 ul from each dilution was plated onto 100 × 15 mm PDA plates with three replicates. The number of CFU was estimated after 24 h incubation at room temperature.

2.3 Sugar beet plants

This study was conducted in a greenhouse (Argus Control Systems, Ltd.; British Columbia, Canada) of the Agricultural Experiment Station of North Dakota State University in Fargo, ND, USA. Three seeds of Fusarium-susceptible variety Maribo 409 (Niehaus, 2015) were planted in 10 × 10 × 12 cm plastic pot (T. O. Plastic Inc.; Clearwater, MN, USA) filled with Sunshine Mix #1 peat (Sun Gro Horticulture Ltd.; Alberta, Canada). One teaspoon of Osmocote 15-9-12 (3-4 months' formula) (Everris NA Inc., Dublin, OH, USA) fertilizer was added and mixed to each pot before seeding. One-week after planting, seedlings were thinned to one plant per pot. Greenhouse conditions were set to an average temperature of 24°C and 16-h photoperiod. Plants were watered as needed. Three-week old sugar beet plants (at 4-leaf stage) were used for inoculation.

To identify the most effective alternative inoculation method, five inoculation methods - the conventional standard method (root-dipping), drench without injury (drenching), drench with injury (cutting), *Fusarium* colonized barley seeds placed next to sugar beet plants (barley seed to root), and *Fusarium* colonized barley seeds placed next to sugar beet seeds at planting (barley to seed) were evaluated. After inoculation, all plants were kept in the greenhouse environment set at a temperature of 24°C and 16-h photoperiod, and watered as needed. There were six replicates

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for each isolate. This experiment was performed using a completely randomized design (CRD) two times.

2.4 Inoculation methods

Root-dipping (root-dipping). Three-week old plants were carefully removed from their pots. Roots were washed with distilled water, dried with tissue paper, and soaked in a *Fusarium* spore suspension (5×10^4 spores/ml) for 8 min (Hanson and Hill, 2004). A 1% CMC medium in distilled water was used as a control. After inoculation, plants were transplanted into wet plastic pots as described above. Old-yellow leaves were removed three days after inoculation (Hanson and Hill, 2004).

Drenching without injury (drenching). Inoculation was conducted by pouring 20 ml of *Fusarium* spore suspension (5×10^4 spores/ml) uniformly across the soil surface of pots containing one three-week old plant each (Maitlo et al., 2016). Control pots had 1% CMC medium in distilled water poured instead of spore suspension.

Drenching with injury (cutting). To injure three-week old sugar beet roots, two longitudinal cuts about 10 cm deep were made about 1.3 cm away from opposite sides of each root using a knife sterilized in 75% ethanol. These two cuts were parallel to each other. Inoculation was performed the same way as drench inoculation without injury. The control plants were inoculated with 1%CMC medium in distilled water.

***Fusarium* colonized barley seeds placed next to sugar beet plants (barley to root).** Inoculation was conducted by placing one *Fusarium* colonized barley seed 1 cm away from root and 2 cm deep from soil surface and then covered with Sunshine Mix #1 peat for each sugar beet plant (Liu and Khan, 2016). A sterilized barley seed without *Fusarium* infection was placed beside each control plant.

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***Fusarium* colonized barley seeds placed next to sugar beet seeds at planting (barley to seed).** For this inoculation method, 28 ×12 × 12 cm plastic trays were used, and fertilizer was added to potting soil as previously described. Ten sugar beet seeds were planted in 2 cm deep furrows and one *Fusarium* colonized barley seed was placed 1 cm to the side of each sugar beet seed and each covered with Sunshine Mix #1 peat (Liu and Khan, 2016). A sterilized barley seed that was not inoculated with the pathogen was placed beside each control seed.

Foliar and root disease symptom evaluation. Disease evaluation was based on Fusarium yellows and Fusarium yellowing decline symptoms. The severity scale used to assess foliar disease symptoms in the study was as follows: 0 = no disease; 1 = leaves wilted, small chlorotic areas on lower leaves, most of leaf green; 2 = leaves showing interveinal yellowing; 3 = leaves with 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; 5 = plant death (Hanson et al., 2009). Disease severity ratings were taken every week for five weeks after inoculation.

Five weeks after inoculation, plants were carefully removed from pots, washed under tap water, and roots were longitudinally cut to check for discoloration within the vascular system. The severity scale used for root symptom rating was as follows: 0 = no internal browning; 1 = slight internal browning, usually at the tip of the tap root; 2= moderate to severe internal browning of the entire tap root; and 3 = severe internal browning extending from the tap root into the lower stem above the soil line (Rowe, 1980).

2.6 Data analysis

2.6.1 ANOVA-type statistic test

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Levene's test was first used to determine whether the data sets for disease severity had homogeneous variances and could be combined for analyses. Then data was analyzed by non-parametric method, using the rank and mixed procedures of SAS (Version 9.4, SAS Institute Inc.; Cary, NC, USA). Data from foliar and root symptoms were ranked separately using the Rank procedure and ANOVA-type statistic (ATS) analysis was conducted using the mixed procedure. For foliar data, the significance of the main effect of isolate, inoculation method, timing of observation and their interactions were evaluated. For root data, the main effect of isolate, inoculation method and their interactions were evaluated. SAS macros F2_LD_F1 and LD_CI were used to calculate relative treatment effects and their 95% confidence intervals (Shah and Madden, 2004).

2.6.2 Data transformation

To assess the rate of foliar symptom severity progress through time after inoculation, foliar symptom severity scale at each observation point was normalized to the maximum scale of 5 expressed as a percent. We called this transformed symptom severity as normalized symptom severity. For example, if a plant was evaluated with a leaf symptom scale of 2, the normalized symptom severity (NS_L%) was (2÷5) x 100, which came to be 40%.

The root symptom severity was assessed at the end of the experiments and the maximum root score of 3 was used to normalize the data. So, if the root symptom was scored 2 for a plant, the normalized root symptom severity expressed as a percent (NS_R%) was (2÷3) x 100, which came to be 66.67%.

The normalized symptom severity values of NS_L% and NS_R% observed at the end of the experiment were subjected to analysis ~~and~~ of variance. Mean normalized symptom severity value

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of each alternative inoculation was tested against the mean value of the standard root-dipping method as a control using Dunnett's method.

2.6.3 Fitting Gompertz equation

The mean normalized leaf symptom severity value from each observation time point under each inoculation method in two experiments was fitted to the Gompertz model expressed as the following form (Tjørve and Tjørve, 2017):

$y = A * \exp(-\exp(-b * (DAI - T_i))$ Eq. 1

in which y is the normalized leaf symptom severity value expressed as a percent (i.e. NSL%); DAI is the days after inoculation; A is the asymptotic value (i.e. the maximum relative disease severity); b is the slope curvature parameter controlling the rate at which the disease severity progresses with time; T_i is the infection point of days after inoculation at which the slope is steepest (i.e. the rate of increase in disease is the highest). This model was fitted separately for the two isolates. Parameters were obtained using the nonlinear least squares method with the NLIN procedure of SAS (Version 9.4, SAS Institute Inc.; Cary, NC, USA).

3. Results

The two runs of data for this study were combined because their homogeneity test for variance was not significantly different ($p > 0.67$). The negative controls for each inoculation method were without foliar or root symptoms of Fusarium yellows or Fusarium yellowing decline and were not included in data analysis.

Table 1 showed ANOVA-type statistics for sugar beet disease severity based on foliar symptom observation. Inoculation methods and timing of observations resulted in significantly different foliage disease severity ($p < 0.001$). Isolates differed significantly in foliage disease symptom severity ($p < 0.001$). *F. oxysporum* f. sp. *betae* F-19 caused higher severity score than

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F. secorum 784-12-4. Importantly, the two-way and three-way interactions were all significant. So, an appropriate time point was chosen to assess the foliage disease severity between isolates among different inoculation methods. The ANOVA-type statistics for root symptom severity was given in Table 2. Again, the root symptom severity significantly differed between the two isolates and among the five inoculation methods. Interaction of isolate with inoculation method on root symptom scores was also present ($p < 0.001$).

Table 3 shows foliar disease severity for all treatments of inoculated sugar beet at 7, 14, 21, 28 and 35 DAI (days after inoculation). The Gompertz model describing progress of normalized foliage symptom severity was shown in Fig. 1 while the respective parameter estimates were given in Table 4. With majority of the inculation methods, leaf symptoms caused by two *Fusarium* species were first observed at 14 DAI, except for the treatments with *F. secorum* using barley to root where symptoms were first observed at 21 DAI. Root-dipping, barley to root, and barley to seed had the similar high rate of the increase in foliar symptoms. For the barley to root inoculation method, the use of *F. secorum* resulted in significantly lower disease development than *F. oxysporum* f. sp. *betae*.

Table 5 showed that root disease severity for all the treatments of inoculated plants at 35 DAI. Among all the treatments, root-dipping and barley to seed with both species, and barley to root with *F. oxysporum* f. sp. *betae* resulted in the highest similar disease severities. However, *F. secorum* 784-12-4, via barley to root inoculation, did not induce a similar root symptom like *F. oxysporum* f. sp. *betae*. Cutting with *F. oxysporum* f. sp. *betae* was not significantly different from root-dipping and barley to seed methods with a lower infection on sugar beet roots but the disease severity was low with *F. secorum* 784-12-4. Drenching induced root symptoms, but was inconsistent.

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Normalized foliage (i.e. NS_L%) and root symptom (i.e. NS_R%) severities caused by each isolate at 35 DAI using standard root-dipping inoculation method were compared with those using each of the four alternative inoculation methods from analysis of variance. Regarding foliage symptom severity by *F. secorum* 784-12-4, barley to seed inoculation did not differ significantly from standard root-dipping inoculation ($p > 0.05$) whereas the standard root-dipping method resulted in significantly higher NS_L% than the other three alternative methods (Supplementary ~~Table 6~~Table S1). However, regarding foliage symptom severity by *F. oxysporum* f. sp. *betae* F-19, the standard root-dipping method did not cause significantly higher NS_L% than any of the four alternative inoculation methods ($p > 0.05$) (~~Table 6~~Table S1). Similarly, the normalized root symptom severity (i.e. NS_R%) was not significantly different between root-dipping and barley to seed inoculation method with *F. secorum* 784-12-4 nor significantly different between root-dipping and all four alternative inoculation methods (Supplementary ~~Table 7~~Table S2).

3. Discussion

The standard root-dipping method was the most effective inoculation method for both *Fusarium* species inoculation on sugar beet. Root-dipping method included soaking seedlings in spore suspension followed by transplanting. During this process, spores could directly get in contact with the damaged root system and lead to pathogens entering the vascular system through wounds. Therefore, root-dipping method allowed the pathogens avoid resistance mechanisms at the root epidermal level (Eynck et al., 2009; Michielse and Rep, 2009). Studies showed *F. oxysporum* f. sp. *betae* could directly penetrate root epidermis after forming and accumulating net-like hyphae on the surface of root tips , and then colonize tissues intracellularly and intercellularly (Bishop and Cooper, 1983; Czymmek et al., 2007; Mendgen et al., 1996; Van

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Peer and Schippers, 1992). This also explains why drench inoculation without injury (drenching) and with injury (cutting) caused the same level of disease symptom severity. However, these two inoculation methods caused significantly lower foliage disease severity than the standard root-dipping method with *F. secorum* 784-12-4. Spore distribution in soil is limited by spore morphology and electrical charge, and by soil physical features (Hepple, 1960; Wallace, 1978). Gracia-Garza and Favel's (1998) study showed spores were unevenly distributed in soil, and CFU at 0-2 cm depth were 10-times higher than at 8-10 cm depth. The low disease severity observed on drench treatments in our study may be due to the majority of the spores applied in the drench treatment remained on the surface and the top 2 cm of the soil reduced the chance of spores getting in contact with sugar beet roots and thus resulted in low disease severity.

Barley-based inoculum has been used to study the effect soil borne pathogens like *Rhizoctonia solani* on sugar beet before (Gaskill, 1968; Kirk et al., 2008; Noor and Khan, 2014). In our study, *Fusarium*-colonized barley inoculum placed by the sugar beet seed at planting time caused the highest disease severity with symptoms being observed as early as 7 DAI compared with the 14 DAI for standard root-dipping method and did not allow for distinction between isolates. However, placing the *Fusarium*-colonized barley seeds by the roots of sugar beet plants, *F. secorum* caused significantly lower disease severity with delayed onset of symptoms (21 DAI) compared with *F. oxysporum* f. sp. *betae* (14 DAI). Also, during this investigation, *F. oxysporum* f. sp. *betae* (F-19) grew faster and more abundantly on barley than *F. secorum* (784-12-4). CFU for *F. oxysporum* f. sp. *betae* (F-19) was 4.8×10^5 CFU/barley, which was 2.6-times higher than the CFU for *F. secorum* (784-12-4) (Data not shown). Plant stage also had an effect on sugar beet disease severity and younger plants were more susceptible than older plants.

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Burlakoti et al. (2012) reported that different *Fusarium* species could have similar foliar symptoms at 60 DAI, but when evaluating the diseased roots, the more-virulent isolates resulted in more vascular discoloration than the less-virulent ones. In this study, foliar symptoms were evaluated by using a scale to record the yellowing response at 0, 7, 14, 21, 28 and 35 DAI contributing to disease severities for each *Fusarium* isolate. This evaluation method for foliar symptoms caused by *Fusarium* species could be reliable, because both foliar (Table 3) and root (Table 4) evaluations indicated that *F. oxysporum* f. sp. *betae* (F-19) induced significantly higher disease severity than *F. secorum* isolate (784-12-4). Burlakoti et al. (2012) reported that *F. secorum* was more aggressive than *F. oxysporum* f. sp. *betae* on sugar beet. However, the specific isolate number of *F. secorum* was unknown. Since *F. secorum* was a relatively new species (Rivera et al., 2008), the differentiation in pathogenicity and virulence among its isolates was still unclear. Given the fact by Hill et al. (2011) that *F. oxysporum* f. sp. *betae* (F-19) was evaluated as highly pathogenic to sugar beet, *F. oxysporum* f. sp. *betae* (F-19) could be more aggressive than the specific *F. secorum* isolate 784-12-4.

In conclusion, this study evaluated artificial inoculation methods to induce *Fusarium* diseases on sugar beet in greenhouse conditions. The results showed both root-dipping and barley to seed were effective inoculation methods with both isolates when symptoms were assessed at 35 days after inoculation that could be used for *Fusarium* study on sugar beet. However, for large scale sugar beet germplasm resistant selection, root-dipping method is time consuming, labor intensive, and impractical for field study since this method requires transplanting after inoculation. Therefore, the barley to seed can be an alternative inoculation method that could be used for *Fusarium* study on sugar beet.

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Table 1. Test statistics for the effect of five different inoculation methods and two *Fusarium* species on foliar disease severity of sugar beet at 7, 14, 21, 28, and 35 DAI[a].

Effect	ANOVA -type statistic (ATS)			
	df_N[b]	df_D[c]	ATS	*P*-value
Isolate	1	1	28.78	<0.0001
Inoculation Method	3.35	1	27.98	<0.0001
Isolate × Inoculation Method	3.35	4.09	5.54	0.0005
Time	2.85	1	400.14	<0.0001
Isolate × Time	2.85	1	11.03	<0.0001
Inoculation Method × Time	7.52	1	9.15	<0.0001
Isolate × Inoculation Method × Time	7.52	1	3.02	0.0028

[a]DAI=days after inoculation
[b]df_N=numerator degrees of freedom.
[c]df_D=denominator degrees of freedom.

Table 2. Test statistics for the effect of five different inoculation methods and two *Fusarium* species on disease severity of sugar beet root at 35 DAI[a].

Effect	ANOVA-type statistic (ATS)			
	df_N[b]	df_D[c]	ATS	*P*-value
Isolate	1	47.9	49.54	<0.0001
Inoculation Method	2.24	47.9	24.6	<0.0001
Isolate × Inoculation Method	2.24	47.9	10.85	<0.0001

[a]DAI=days after inoculation
[b]df_N=numerator degrees of freedom.
[c]df_D=denominator degrees of freedom.

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Table 3. Effect of five different inoculation methods and two *Fusarium* species on foliar disease severity of sugar beet at 7, 14, 21, 28, and 35 DAI[a].

Inoculation method	DAI	MDS[b]		MR[c]		REDS[d]		95%CI[e]	
		F. oxysporum	*F. secorum*	*F. oxysporum*	*F. secorum*	*F. oxysporum*	*F. secorum*	*F. oxysporum*	*F. secorum*
Dipping	7	0.00	0.00	122.50	122.50	0.203	0.203	0.187-0.220	0.187-0.220
Dipping	14	4.00	3.00	373.33	332.66	0.621	0.554	0.562-0.677	0.510-0.596
Dipping	21	5.00	4.50	487.08	452.99	0.811	0.754	0.752-0.858	0.693-0.806
Dipping	28	5.00	5.00	501.75	482.24	0.835	0.803	0.801-0.865	0.750-0.846
Dipping	35	5.00	5.00	501.75	491.98	0.835	0.819	0.801-0.865	0.774-0.857
Drenching	7	0.00	0.00	122.50	122.50	0.203	0.203	0.187-0.220	0.187-0.220
Drenching	14	0.00	0.00	146.63	134.60	0.244	0.223	0.196-0.300	0.186-0.266
Drenching	21	1.00	0.00	240.40	210.57	0.400	0.350	0.295-0.516	0.257-0.456
Drenching	28	2.00	1.50	328.01	272.01	0.546	0.452	0.403-0.681	0.366-0.542
Drenching	35	3.50	3.00	402.30	308.44	0.670	0.513	0.575-0.753	0.424-0.601
Cutting	7	0.00	0.00	122.50	122.50	0.203	0.203	0.187-0.220	0.187-0.220
Cutting	14	0.00	0.00	134.59	149.15	0.223	0.248	0.186-0.267	0.195-0.309
Cutting	21	3.50	1.00	323.36	257.28	0.538	0.428	0.396-0.674	0.336-0.526
Cutting	28	5.00	3.00	367.30	280.68	0.611	0.467	0.434-0.762	0.378-0.558
Cutting	35	5.00	3.00	438.55	307.42	0.730	0.512	0.590-0.834	0.414-0.608
Barley to root	7	0.00	0.00	122.50	122.50	0.203	0.203	0.187-0.220	0.187-0.220
Barley to root	14	2.50	0.00	304.56	122.50	0.507	0.203	0.394-0.619	0.187-0.220
Barley to root	21	5.00	0.00	435.87	176.48	0.726	0.293	0.613-0.814	0.223-0.376
Barley to root	28	5.00	1.00	467.65	236.03	0.778	0.393	0.715-0.830	0.313-0.479
Barley to root	35	5.00	3.00	496.93	334.72	0.827	0.557	0.775-0.869	0.518-0.596

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Table 3. Effect of five different inoculation methods and two *Fusarium* species on foliar disease severity of sugar beet at 7, 14, 21, 28, and 35 DAI[a] (continued).

Inoculation method	DAI	MDS[b]		MR[c]		REDS[d]		95%CI[e]	
		F. oxysporum	*F. secorum*	*F. oxysporum*	*F. secorum*	*F. oxysporum*	*F. secorum*	*F. oxysporum*	*F. secorum*
Barley to Seed	7	0.00	0.00	122.50	122.50	0.203	0.203	0.187-0.220	0.187-0.220
Barley to Seed	14	0.50	2.00	241.33	280.38	0.401	0.466	0.275-0.543	0.333-0.604
Barley to Seed	21	5.00	4.50	454.67	357.61	0.757	0.595	0.629-0.850	0.425-0.744
Barley to Seed	28	5.00	5.00	511.50	450.06	0.852	0.749	0.834-0.867	0.632-0.837
Barley to Seed	35	5.00	5.00	511.50	493.79	0.852	0.822	0.834-0.867	0.758-0.871

[a]DAI=days after inoculation

[b]MDS=median disease rating. Disease severity was evaluated every week for five weeks based a 0 to 5 scale: 0 (no disease), 1 (leaves wilted, small chlorotic areas on lower leaves, most of leaf green), 2 (leaves showing interveinal yellowing), 3 (leaves with 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), 5 (plant death).

[c]MR=mean rank

[d]REDS=relative effect of disease severity

[e]95% CI=upper-lower values of 95% confidence interval (CI) of relative effect.

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Table 4. Effect of five different inoculation methods and two *Fusarium* species on root disease severity of sugar beet at 35 DAI[a].

Inoculation method	MDS[b]		MR[c]		REDS[d]		95%CI[e]	
	F. oxysporum	*F. secorum*	*F. oxysporum*	*F. secorum*	*F. oxysporum*	*F. secorum*	*F. oxysporum*	*F. secorum*
Dipping	3	3	80.50	80.50	0.667	0.667	0.641-0.691	0.641-0.691
Drenching	3	2	56.04	32.50	0.463	0.267	0.337-0.595	0.177-0.391
Cutting	3	2	63.63	27.04	0.526	0.221	0.392-0.656	0.149-0.327
Barley to root	3	1	80.50	23.29	0.667	0.190	0.641-0.691	0.122-0.302
Barley to Seed	3	3	80.50	80.50	0.667	0.667	0.641-0.691	0.641-0.691

[a]DAI=days after inoculation
[b]MDS=median disease rating. Sugar beet plants were hand harvested at 35 DAI and root disease severity was rated with a 0 to 3 scale: 0 (no internal browning), 1 (slight internal browning, usually at the tip of the tap root), 2 (moderate to severe internal browning of the entire tap root), and 3 (severe internal browning extending from the tap root into the lower stem above the soil line).
[c]MR=mean rank
[d]REDS=relative effect of disease severity
[e]95% CI=upper-lower values of 95% confidence interval (CI) of relative effect.

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Table 5. Estimate values for parameters in Gompertz equation (i.e. Eq. 1) for each combination of two isolates with five inoculation methods

Isolate	Inoculation method	A[a]	b[b]	T_i[c]	R^2[d]
F. secorum 784-12-4	Dipping	95.992	0.314	11.984	1.000
	Drenching	55.084	0.148	20.738	0.974
	Cutting	45.739	0.267	16.892	0.998
	Barley to root	100.000	0.115	30.243	0.984
	Barley to seed	100.000	0.090	15.815	0.974
F. oxysporum f. sp. betae F-19	Dipping	98.038	0.388	11.213	1.000
	Drenching	93.697	0.115	23.710	0.999
	Cutting	72.668	0.334	17.474	0.986
	Barley to root	95.478	0.209	12.578	0.993
	Barley to seed	100.000	0.152	13.226	0.999

[a]A=the asymptotic value (i.e. the maximum relative disease severity)
[b]b=the slope curvature parameter controlling the rate at which the disease severity progresses with time
[c]T_i=the infection point of days after inoculation at which the slope is steepest
[d]Coefficient of determination which was the proportion of the variance in the normalized relative disease severity that was predictable from the number of days after the inoculation.

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~~**Table 6**~~**Table S1**. Normalized foliage disease symptom severity (i.e. $NS_L\%$) from root-dipping method in comparison with four alternative inoculation methods at 35 DAI[a].

Isolate	Root-dipping[b] versus	Severity difference	Significant at 5% level
F. secorum 784-12-4	Barley to seed	15.840	NS
	Barley to root	38.330	*
	Drenching	46.670	*
	Cutting	50.000	*
F. oxysporum f. sp. betae F-19	Barley to seed	1.230	NS
	Barley to root	1.670	NS
	Drenching	26.670	NS
	Cutting	20.000	NS

[a]DAI=days after inoculation
[b]Mean $NS_L\%$ with root-dipping method at 35 DAI was 96.67% with *F. secorum* 784-12-4 and 98.33% with *F. oxysporum f. sp. betae* F-19.

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~~Table 7~~Table S2. Normalized root disease symptom severity (i.e. NS_R%) from root-dipping method in comparison with four alternative inoculation methods at 35 DAI[a].

Isolate	Root-dipping[b] versus	Severity difference	Significant at 5% level
F. secorum 784-12-4	Barley to seed	12.256	NS
	Barley to root	55.556	*
	Drenching	44.444	*
	Cutting	50.000	*
F. oxysporum f. sp. betae F-19	Barley to seed	1.320	NS
	Barley to root	0.000	NS
	Drenching	0.000	NS
	Cutting	19.440	NS

[a]DAI=days after inoculation

[b]Mean NS_R% with root-dipping method at 35 days after inoculation was 100% with *F. secorum* 784-12-4 and 100% with *F. oxysporum f. sp. betae* F-19.

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List of Figures

Figure 1. Normalized foliage symptom severity (i.e. NS_L%) progress through time post inoculation with five inoculation methods with *F. secorum* 784-12-4. (Fig. 1a) and with *F. oxysporum f. sp. betae* F-19 (Fig. 1b). Estimated parameter values are shown in Table 5 for the fitted Gompertz model.