RESPONSE OF SUGAR BEET CULTIVARS TO THREE SPECIES OF FUSARIUM ASSOCIATED WITH SUGAR BEET YELLOWS

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Fusarium yellows caused by *Fusarium oxysporum* f. sp. *betae* was first described by Khan et al. (Khan et al., 2003) from the Red River Valley (RRV) of North Dakota (ND) and Minnesota (MN). Burlakoti et al., 2007 reported that isolates of *F. sambucinum*-like species were more pathogenic compared to *F. oxysporum*, and *F. graminearum* isolates collected from Sabin, Georgetown , and Fossum, MN. Since 2003, the disease recently has emerged as a serious problem for growers in the RRV of ND and MN (Khan et al., 2003).

Identification of cultivars with resistance for *Fusarium* species present in the RRV provides useful information for breeders as well as growers. In ND and MN, field screening can only be done in the summer and trials need to be done at multiple sites where the disease is present and where weather does not adversely impact the trials. It will be useful to have a greenhouse methodology that accurately determines Fusarium tolerant and susceptible cultivars using pathogenic Fusarium isolates representative of the region. Researchers can then focus on cultivars that are more tolerant to Fusarium in field trials. The objectives of this research were (i) to determine in the greenhouse the tolerance of sugar beet cultivars to three different *Fusarium* species prevalent in the RRV area, and (ii) to determine the tolerance of sugar beet cultivars at a known disease site and compare response of the cultivars in the greenhouse and field conditions.

MATERIALS AND METHODS

Greenhouse Study

Plant material and growing conditions. Eight cultivars, namely Van der Have 46177, Van der Have 66561, Beta 4818R, Beta 4797R, Seedex Magnum, Hilleshog 2463Rz, Crystal 820 and Crystal R434, with different levels of susceptibility to Fusarium yellows were grown in the greenhouse for 38 days. Seeds were sown in 3.8 cm diameter x 21 cm deep cone-tainers (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 cubic m of soil and grown for 38 days. Plants were grown in a greenhouse under fluorescent light (16-h photoperiod) and 27±2°C.

Isolates and inoculum Preparation. Eight of the most virulent isolates, four *F. sambucinum*-like (Fsl 42, Fsl 36, Fsl 45 and Fsl 2), two *F. oxysporum* (Fo 15 and Fo 16) and two *F. graminearum* (Fg 12 and Fg 19), determined in a pathogenicity study (Burlakoti et al., 2007), were selected and used to screen the cultivars independently, under greenhouse conditions. Hyphal material and spore were harvested by flooding the 10 day old culture plates with five ml of sterile distilled water and scraping the plates with a sterile bent glass rod. Spore concentration was determined by a hemocytometer and adjusted to 40,000 macroconidia per ml. Two drops of polyoxyethylene-20-sorbitan monolaurate (Tween 20) (Sigma Chemical Co. St. Louis, MO) was added per 100 ml of distilled water before autoclaving to facilitate adherence and the even distribution of inoculum.

Inoculation and disease assessment. Inoculation with the isolates was done on 38 days old sugar beet seedlings by bare-root inoculation method described by Hanson and Hill (2004). Roots were inoculated with sterile distilled water as a control. Plants were then transplanted immediately to fresh cone-tainers, as previously described, and then kept for two days in a growth chamber where the temperature was 21 ±2 ^{o}C for two days to reduce transplanting shock. The plants were subsequently transferred into the greenhouse at 27 ±2 ^{o}C. Natural light was supplemented with fluorescent light. Space was kept in between cones and trays to avoid cross contamination. Three days after transplanting, the oldest two or three leaves were removed to avoid confusing between yellowness on leaves due to Fusarium, senescence or transplanting shock. Plants were grown in the green house for 60 days after inoculation (DAI). Preliminary study indicated that some isolates did not caused vascular discoloration after 35 DAI. As such, inoculated plants were evaluated for 60 DAI in this study. Plants were watered daily with care so that there was no splashing of soil or water between different treatments. Plants were independently examined and rated twice weekly for foliar symptoms using a rating scale of 0-4 similar to Hanson and Hill (2004) with slight modifications where, 0= healthy plants, 1= plants stunted and wilted with few yellowed leaves (1-24%); 2= chlorosis and necrosis of leaves (25-59%); 3= crown becoming dried and

brown to black in color, leaves dying (60-89%); and 4= death of entire plant (90-100%). Re-isolation of pathogen was done for confirmation. Plants that were infected and died before 60 days were harvested for re-isolation once they die. The experiment was designed as a randomized complete block with two factors, *Fusarium* species and cultivars. Each treatment comprised of six plants with three replicates. The experiment was conducted twice.

Field Study

The field trial was conducted at Sabin, MN in 2006 and 2007, in a field with a history of severe Fusarium yellows. Eight commercial cultivars, the same as used in the greenhouse study, were planted using a John Deere MaxEmerge 2 planter into plots 3.35 m wide (6 row plots with 56 cm row spacing) and 7.62 m long on May 18, 2006 and May 11, 2007. Fertilization was done according to standard recommendation for sugar beet. Plots were kept weed free using micro-rates of herbicides recommended for sugar beet (Khan, 2006) and hand-weeding. The experimental design was a randomized complete block with four replicates. Plots were naturally infected. Plots were rated for foliar symptoms using the 0-4 scale (as in the greenhouse) at 10 days interval. The middle-two rows of plots were harvested on September 11, 2006 and September 13, 2007 for yield and quality determinations. Quality analysis of samples was done at American Crystal Sugar Company Quality Tare Laboratory, East Grand Forks, MN. Three infected roots from each of the middle two rows were sampled after harvesting to confirm the pathogen.

Data analysis. Disease severity and AUDPC of each species among the isolates inoculated were averaged and used for analysis. Foliar ratings were converted to percentages, using the mid-point rule (Campbell and Madden, 2006), and the foliar severity value for each rating category was used to calculate AUDPC using;

$$\text{AUDPC} = \sum_{i=1}^{n-1} [(X_{i+1}+Y_i)/2] (t_{i+1}-t_i)$$

where X_i = Percent of foliar symptom severity at the i^{th} observation, t_i = time (days between ratings) at the i^{th} observation, and n = total number of observation. Cultivars with mean disease severity less than 1.0 were considered as tolerant.

Analysis of variance (ANOVA) to determine the effects of treatments on AUDPC was performed using SAS version 9.1 (Statistical Analysis System, Cary, NC). Least significance difference (LSD) at $P = 0.05$ was calculated to compare differences in mean AUDPC among the cultivars in the greenhouse and field conditions. LSD at $P = 0.05$ was also calculated to compare differences in mean stand count at harvest, recoverable sucrose per acre (RSA) and sucrose concentration among the cultivars in the field trials. Correlation analysis was done to determine the association between the greenhouse and field trials based on standardized AUDPC. Standardized AUDPC was calculated using;

Standardized AUDPC = AUDPC / N
where, N = number of days plants were rated.

RESULTS AND DISCUSSION

Greenhouse tests. Varietal response to Fusarium yellows was described based on disease severity and AUDPC. Means for disease severity (on a scale of 0-4) by *F. oxysporum*, *F. graminearum* and *F. sambucinum*-like species for the cultivars of sugar beet ranged from 0 to 3.8 (Table 1). *F. sambucinum*-like species was more pathogenic to sugar beet cultivars followed by *F. oxysporum* and *F. graminearum*. AUDPC caused by *F. sambucinum*-like species was also higher than *F. oxysporum* followed by *F. graminearum* (Table 2). There were significant differences in AUDPC among the cultivars and significant differences in AUDPC caused by the different Fusarium species (Table 2). Van der Have 46177 was most susceptible to Fusarium with the highest AUDPC. Four cultivars, Crystal R434, Crystal 820, Van der Have 66561 and Beta 4797R were consistently more tolerant than Beta 4818R, Hilleshog 2463Rz and Seedex Magnum based on AUDPC. There was no disease on plants inoculated with sterile distilled water.

Field tests. Varietal response to Fusarium yellows was described based on disease severity, AUDPC and RSA. Cultivars that were more tolerant in the greenhouse study were generally more tolerant in the field study. Van der Have 46177 was a known susceptible cultivar (Niehaus, 2006) and was used as check to determine the presence and severity of disease. As expected, Van der Have 46177 had the highest disease severity, AUDPC and lowest yield. Mean disease severity of cultivars was significantly different with Van der Have 46177 being the most susceptible and Crystal R434, Beta 4797R, Crystal 820, and Van der Have 66561 being more tolerant than Beta 4818R, Hilleshog 2463Rz and Seedex Magnum (Table 3). AUDPC was also lower

for the more tolerant cultivars compared to the less tolerant cultivars. Plant stand was lowest in the most susceptible Van der Have 46177; however, plant stand for each cultivar was not consistent over the years, although they were higher in 2007 compared to 2006. Sucrose concentration (% S) was always lowest in the most susceptible Van der Have 46177 compared to the other seven cultivars evaluated. Van der Have 46177 also had the lowest recoverable sucrose per acre in both years of the study. The more tolerant Crystal R434, Beta 4797R, Crystal 820, and Van der Have 66561 consistently had higher RSA than the less tolerant Beta 4818R, Hilleshog 2463Rz and Seedex Magnum. In 2006, RSA for all cultivars were higher compared for the same cultivars 2007, probably because of better growing conditions in 2006. In the field, more tolerant cultivars which had lower disease severity would have had less damage to leaves and less plugging of vascular system by mycelia and microconidia. As such, more tolerant plants ultimately had more photosynthetic area which resulted in higher RSA compared to less tolerant cultivars.

Correlation analysis. Significant correlations (r = 0.98 and 0.99 in 2006 and 2007, respectively) occurred between the standardized AUDPC for the eight cultivars in the field and greenhouse tests. Significant correlations (r = 0.93 and 0.95 in 2006 and 2007, respectively) were also found between the highest disease severity and AUDPC under field conditions.

Since the greenhouse and field data are consistent, it will strongly support the use of greenhouse screening by bare-root inoculation to determine tolerance level to the most pathogenic and virulent isolates of species causing Fusarium yellows. Seed Companies usually need to test large number of accessions before releasing commercial cultivars. It could be economical and efficient to use a greenhouse bioassay to screen accessions for tolerance to Fusarium yellows so that only those with acceptable levels of tolerance could be tested in field trials.

LITERATURE CITED

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Table 1. The highest disease severity caused by *F. oxysporum*, *F. graminearum* and *F. sambucinum*-like species during 60 DAI on sugar beet cultivars in greenhouse conditions.

Cultivars	**Disease Severity**			
	F. oxysporum	***F. graminearum***	***F. sambucinum*-like species**	**Control**
Van der Have 46177	3.4 b	1.0 f	3.8 a	0 j
Seedex Magnum	1.8 cd	0.3 i	1.8 c	0 j
Beta 4818R	1.6 de	0.3 i	1.7 cde	0 j
Hilleshog 2463Rz	1.6 de	0.3 i	1. 6 e	0 j
Crystal 820	0.8 gh	0 j	0. 9 fg	0 j
Van der Have 66561	0.7 h	0 j	0.9 fg	0 j
Beta 4797R	0.1 j	0 j	0.4 i	0 j
Crystal R434	0.1 j	0 j	0.1 j	0 j
CV %			22.33	

Table 2. Area under the disease progress curve (AUDPC) of Fusarium yellows caused by *F. oxysporum*, *F. graminearum* and *F. sambucinum*-like species during 60 DAI on sugar beet cultivars in greenhouse conditions.

	Area under the disease progress curve[z]			
Cultivars	***F. oxysporum***	***F. graminearum***	***F. sambucinum*-like species**	**Control**
Van der Have 46177	2580.13b	360.3 g	3365.42 a	0 n
Beta 4797R	279.9 hi	0 n	282.9 h	0 n
Crystal 820	98.61 k	0 n	56.08 l	0 n
Seedex Magnum	976.1 c	43.75 lm	875 e	0 n
Crystal R434	19.44 mn	0 n	18.33 mn	0 n
Van der Have 66561	249.2 ij	0 n	233.0 j	0 n
Hilleshog 2463Rz	867.7 e	38.89 lm	858.7 e	0 n
Beta 4818R	940.2 d	41.35 lm	790.2 f	0 n
CV %		---------- 10.48 ----------		

[z] Results represent a combination of two trials. Numbers followed by the same letter are not significantly different.

Table 3. Mean of disease severity, area under the disease progress curve, stand count, recoverable sucrose per acre and sucrose concentration of eight different cultivars at Sabin, MN, 2006 and 2007[u].

Cultivars	2006					2007				
	DS[v]	AUDPC[w]	SC[x]	RSA[y]	% S[z]	DS[v]	AUDPC[w]	SC[x]	RSA[y]	% S[z]
Van der Have 46177	2.8 a	2815.75 a	40 c	2942 c	12.02 b	2.8 a	2727 a	59.25	1684.2d	12.9 d
Seedex Magnum	1.5 b	715.1 b	60 b	5921 b	13.87 a	1.2 c	806.1 b	70.5	4469.2 bc	15.04 bc
Beta 4818R	1.2 c	673 b	52 b	5543 b	13.02 ab	1.5 b	714.5 b	73	4481.5 bc	15.02 bc
Hilleshog 2463Rz	1.2 c	806.1 b	54 b	5833 b	13.23 ab	1.2 c	673 b	75	3751.6 c	14.76 c
Crystal 820	0.9 d	291 cd	57 b	6100 b	14.25 a	0.8 e	426.4 c	76.5	5441.7 a	16.46 a
Van der Have 66561	0.8 e	426.4 c	53 b	7371 a	14.085a	0.6 f	321.6 cd	100	5276.5 ab	16.25 ab
Beta 4797R	0.6 f	321.6 cd	59 b	6535 ab	13.52 a	0.9 d	291 cd	83	5325.2 ab	16.12 ab
Crystal R434	0.4 g	225.6 d	72 a	7321 a	12.96 a	0.4 g	217.4 d	96.25	5609.9 a	16.05 ab
CV	9.07	17.36	10.13	11.86	7.2	11.73	15.51	50.14	13.53	5.53

[u] Means within a column having same letters are not significantly different ($P = 0.05$).
[v]DS = Highest disease severity during 60 DAI.
[w] AUDPC = Area under the disease progress curve.
[x] SC = Stand count at harvest.
[y]RSA = Recoverable sucrose per acre.
[z] %S = Sucrose concentration.