BASELINE SENSITIVITY OF *FUSARIUM* SPECIES ASSOCIATED WITH SUGAR BEET YELLOWS TO METCONAZOLE, TRITICONAZOLE AND THIABENDAZOLE FUNGICIDES

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Minnesota (MN) and North Dakota (ND) ranked first in sugar beet production in the United States (US) during 2007. The fungal diseases, Cercospora leaf spot, Aphanomyces root rot and Rhizoctonia root and crown rot, are the most important constraints for the sugar beet industry in this region. In 2002, another fungal disease, Fusarium yellows was reported in MN and ND (Khan et al., 2003). Fusarium yellows is starting to become a serious problem particularly for growers in the Moorhead factory district (Windels et al., 2005). Pathogenicity studies (Burlakoti et al., 2007) confirmed that *F. oxysporum, F. graminearum*, and *F. sambucinum*-like species collected from sugar beets with foliar symptoms of Fusarium yellows from fields located in Sabin, Fossum and Georgetown, west-central MN, caused yellows and vascular discoloration of sugar beet seedlings in green house conditions.

Fusarium yellows on sugar beet cause significant qualitative and quantitative yield loss due to poor plant population, growth reduction of plants, and increased impurities in extracted juice (Schneider and Whitney, 1986). There is an urgent need to develop an integrated system for managing Fusarium yellows. Resistance genes for Fusarium yellows of sugar beet have not been identified to date (Francis and Luterbacher, 2003). Fungicides are widely used to control many diseases. However, since Fusarium yellows is a new disease in MN and ND, no previous study on the use of fungicides for controlling Fusarium yellows in sugar beet has been reported in these states. As such, there is a great need to determine the efficacy of fungicides for controlling yellows in sugar beet.

At present, fungicides containing triazoles are the most effective chemical control for *Fusarium* species (Mesterhazy et al., 2003). Thiabendazole (TBZ) [2-(4-thiazolyl)-1H-benzimidazole] is an effective fungicide that controls Fusarium wilt in potato caused by several *Fusarium* species. *In vitro* sensitivity of *Fusarium* species associated with sugar beet to triazoles and TBZ has not been determined. It would be useful to determine sensitivity of *Fusarium* species associated with Fusarium yellows of sugar beet to triazoles and TBZ. The objective of this study was to determine the baseline sensitivity of *F. oxysporum*, *F. graminearum* and *F. sambucinum*-like populations associated with sugar beet in the Red River Valley to metconazole, triticonazole and TBZ.

MATERIALS AND METHODS

Isolate collection and growing condition. Ninety-eight isolates [50 *Fusarium sambucinum*-like, 18 *Fusarium oxysporum*, and 30 *Fusarium graminearum*] isolated from Fusarium yellows diseased sugar beet in 2005, were collected, identified and provided by Dr. Gary A. Secor (Department of Plant Pathology, NDSU).These ninety-eight isolates were collected from Sabin, Georgetown , and Fossum, MN. Twenty five *Fusarium oxysporum* isolates were also collected from sugar beet from Sabin, West Central MN, during 2006. Known TBZ-resistant and TBZ-sensitive isolates of *Fusarium sambucinum* from potato, provided by G. A. Secor, were used as a check in the TBZ-sensitivity *in-vitro* assay and were also grown on metconazole and triticonazole amended plates.

Each isolate was transferred to two petri dishes containing freshly prepared half strength potato dextrose agar (HPDA) and incubated at 23 to 26 °C for five days under 24 h fluorescent light.

Fungicide sensitivity *in vitro* assay. The sensitivity of each isolate to TBZ (Merck and Co., Rahway, NJ), metconazole and triticonazole (BASF, Raleigh, NC) was determined by comparing the radial growth of each isolate on fungicide amended medium to growth in non-amended medium. TBZ was dissolved in dimethylsulfoxide (DMSO) due to higher solubility and to control bacterial growth.

Triticonazole and metconazole were dissolved in sterile distilled water to obtain stock solution of 100µg mL^{-1} and further diluted to 10, 1, 0.1 and 0.01. Each concentration of each fungicide was incorporated into media after the media was autoclaved. The effect of the fungicide on mycelial growth in vitro was determined on half strength home-made PDA medium amended with 0, 0.01, 0.1, 1, 10 and 100 µg mL^{-1} of fungicide.

A 5 mm in diameter mycelial plug, from the margin of a five day old actively growing culture of each isolate, was inverted and transferred to the center of petri dishes (90 mm) with the amended media and the control. Sensitivity was assessed by measuring colony diameter of mycelial growth after 6 days of incubation at room temperature in the dark for *F. graminearum* and after 7 days of incubation for *F. oxysporum* and *F. sambucinum*-like. Two perpendicular measurements of colony diameter, excluding the original plug diameter (5 mm), were obtained from each plate. Within each trial, isolates were replicated twice with two plates per replication at each fungicide concentration. This study was conducted twice. The diameter of each colony on fungicide amended medium relative to the diameter of the colony on non-amended medium was recorded. The relative growth reduction percentage for each fungicide concentration was calculated as follows;

[100-(diameter with fungicide/diameter on control)*100]

EC_{50} values of each isolates were calculated by determining the effective fungicide concentration that inhibited mycelial growth by 50% as previously described (Pasche et al., 2004).

Data analysis. The effective fungicide dose that inhibited radial growth by 50% (EC_{50}) was determined for each isolate-fungicide combination by using the Statistical Analysis System (SAS Institute, Cary, NC). Data from the two experiments were combined for analysis.

RESULTS

All the isolates of *F. oxysporum*, *F. graminearum*, and *F. sambucinum*-like, and TBZ-sensitive isolates were sensitive to the triticonazole, metconazole and TBZ, but the susceptibility varied among the isolates and species. The TBZ-resistant isolates of *F. sambucinum* were resistant to TBZ but sensitive to metconazole and triticonazole. With a few exceptions, higher concentrations of triticonazole and TBZ were required to reduce colony growth by 50% compared to metconazole. The EC_{50} values of metconazole ranged from 0.0058 µg ml^{-1} to 0.080 µg ml^{-1} for *F. oxysporum*, with a mean of 0.038 µg ml^{-1}. The EC_{50} values of triticonazole ranged from 0.007 µg ml^{-1} to 4.238 µg ml^{-1} for *F. oxysporum*, with a mean of 0.508 µg ml^{-1}. Four isolates had EC_{50} values higher than 2.5 µg ml^{-1} for triticonazole. The EC_{50} values of TBZ for *F. oxysporum* ranged from 0.061 µg ml^{-1} to 0.850 µg ml^{-1}, with a mean of 0.567 µg ml^{-1}. Of the three fungicides evaluated, metconazole was the most effective at reducing mycelial growth of *F. oxysporum* at lower rates (Figure 1).

The EC_{50} values of metconazole for *F. graminearum* ranged from 0.006 µg ml^{-1} to 0.080 µg ml^{-1}, with a mean of 0.031 µg ml^{-1}. The EC_{50} value of triticonazole ranged from 0.007 µg ml^{-1} to 4.238 µg ml^{-1} for *F. graminearum*, with a mean of 2.149 µg ml^{-1}. The EC_{50} value of triticonazole for 17 isolates of *F. graminearum* (>50% of isolates) was higher than 2.5 µg ml^{-1}. The EC_{50} value of TBZ ranged from 0.06 µg ml^{-1} to 0.667 µg ml^{-1} for *F. graminearum*, with a mean of 0.537 µg ml^{-1}.

The EC_{50} values of triticonazole for *F. sambucinum*-like ranged from 0.009 µg ml^{-1} to 0.079 µg ml^{-1} for *F. sambucinum*-like, with a mean of 0.0441 µg ml^{-1}. The EC_{50} value of metconazole ranged from 0.007 µg ml^{-1} to 0.084 µg ml^{-1} for *F. sambucinum*-like, with a mean of 0.0187 µg ml^{-1}. The EC_{50} values of TBZ ranged from 0.373 µg ml^{-1} to 0.921 µg ml^{-1} for *F. sambucinum*-like, with a mean of 0.636 µg ml^{-1}. The EC_{50} value of TBZ, triticonazole and metconazole for the known TBZ-resistant *F. sambucinum* isolates were 50.20 µg ml^{-1}, 6.184 µg ml^{-1} and 0.0182 µg ml^{-1}, respectively, and for the known TBZ- sensitive *F. sambucinum* isolates were 0.97 µg ml^{-1}, 0.813 µg ml^{-1} and 0.0619 µg ml^{-1}, respectively. The concentrations of the triazoles required to reduce mycelial growth is most probably due to their intrinsic activity against specific growth stages of the pathogen or action on different target site to inhibit sterol biosynthesis. Metconazole reduced 50% radial growth of all *Fusarium* species at lower concentrations compared to triticonazole and TBZ. The data suggest that metconazole would probably be effective at controlling *Fusarium* species even at low concentrations but triticonazole may need to be used at higher rates. It was interesting to note that baseline *F. oxysporum*, *F. graminearum* and *F. sambucinum*-like isolates were nearly 13, 68, 2.5 times less sensitive to triticonazole than metconazole, respectively. The sensitivity profile of triticonazole was much wider for *F. oxysporum* and *F. graminearum*.

This study illustrated that all the tested fungicides could probably be used for control of soil borne Fusarium but field trials need to be done for confirmation. It should be noted that in field applications, only a portion of the fungicide applied to plant tissue reaches the fungal pathogen, whereas *in vitro* the fungal mycelium is entirely exposed to the chemical present in the medium. As such, higher concentrations of fungicides are needed to control the disease in the greenhouse or field conditions. Moreover, the environment of the greenhouse and field is more warm and humid than ambient condition. Efficacy of disease control is also dependent on the fungicide formulation applied. Tisdale and Lord (1973) found that TBZ uptake was greater from solutions and suspensions than from dusts.

Should metconazole, triticonazole and TBZ provide effective fusarium control in field conditions, they should be used in an alternation program to manage fungicide resistance. The Fungicide Resistance Action Committee (FRAC) classified TBZ as a high risk fungicide for resistance development. Though TBZ is an effective broad spectrum fungicide due to its single mode of action, chances of reduced sensitivity of TBZ is high. Resistance to TBZ has been documented (Hanson et al., 1996). Triazoles are placed in the medium risk group for resistance development. However, resistance was also documented for triazoles (Koller, 1988). As such, field trials need to be done for confirmation, and resistance management strategies will have to be developed and implemented to prolong usefulness of fungicides.

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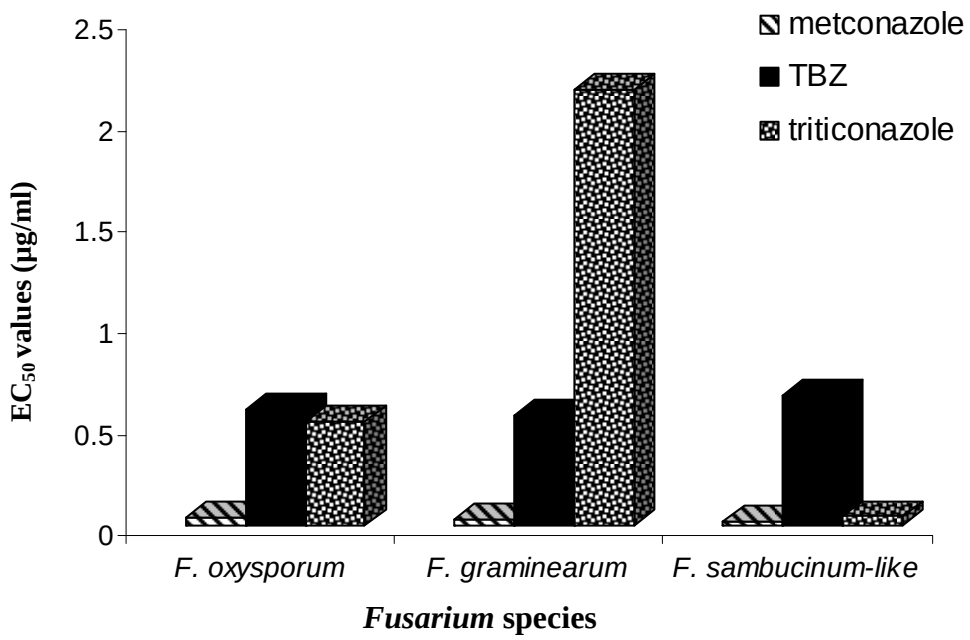

Figure 1. Mean *in vitro* EC_{50} values (µg/ml) of metconazole, TBZ and triticonazole for *F. oxysporum*, *F. graminearum*, and *F. sambucinum*-like isolates.