Identification of Agronomic Practices Associated with the Development of Fusarium Head Blight in Spring Wheat in Southeastern Saskatchewan

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Abstract
Because of the increasing importance of Fusarium head blight (FHB) in western Canada, identification of crop production factors (CPF) associated with the development of this disease would help to devise an effective strategy for its control. From 1999 to 2002, a total of 659 wheat fields were sampled in southeastern Saskatchewan, rated for FHB infection, and categorized according to CPFs used by producers. This study indicated that the environment was the most important factor determining disease development. The effects of the various CPFs on FHB were lower in years with high (2001) and low (1999 and 2002) disease pressure, compared to a year with moderate (2000) disease pressure for this region. The CPFs that affected FHB the most were application of a glyphosate formulation (GF), tillage practice, previously-grown crop, and cultivar susceptibility. GF application in the previous 18 months (or 3 years) was significantly associated with higher FHB levels every year of the study; it was the only CPF in 1999, and one of only two CPFs in 2002, that affected FHB, indicating that its effects were not as influenced by environmental conditions as other CPFs. The relative effects of the other CPFs on FHB were highly variable among years, and were significant in only two of four years. When wheat grown under minimum-till was analysed separately, GF application displayed an even greater effect on FHB than when all tillage systems were analysed together. It is not known if similar effects of GF on FHB would occur in environments different from the ones encountered in this study. Based on the statistically significant and consistent effect of previous GF application on FHB levels throughout the four years of this study conducted in producers’ fields, further research is needed to elucidate the nature of the GF-FHB association and underlying mechanisms.

Introduction
Fusarium Head Blight (FHB), also known as scab or tombstone, has the potential of becoming an important disease of cereal crops in Saskatchewan. Province-wide surveys have indicated that FHB in spring wheat and barley has been increasing in eastern Saskatchewan and spreading westward (e.g. Clear and Patrick, 2003; Fernandez et al., 1999a; 2000; 2001; 2002; Pearse et al., 2003).

There are many Fusarium species that can cause FHB. The most important FHB pathogen in North America is F. graminearum, which produces mycotoxins harmful to humans and livestock. The most commonly found mycotoxin in infected grain is deoxynivalenol (DON). Due to processing problems and potential food safety concerns, tolerance levels for Fusarium damaged kernels (FDK) are very low. For the Canada Western Red Spring class (CWRS), FDK greater than 0.25% by weight will cause downgrading to CWRS No.2, over 1% to CWRS No.3, and over 2% to CWRS No.4, whereas grain with FDK levels of up to 5% would be considered feed (Canadian Grain Commission, 2003). These low tolerance levels could represent significant economic losses to Saskatchewan producers in affected areas.

Because of the increasing importance of FHB in western Canada and its slow spread further westward, it is essential to put in place a comprehensive strategy to stop or reduce the rate of spread of this disease, and to decrease the damage it has been causing in the eastern part of the province, where it is already well established.
To date, there are no wheat cultivars with good resistance to FHB registered in western Canada. The best resistance available is considered intermediate, and is described as ‘fair’ in Saskatchewan Varieties of Grain Crops (2003). Chemical control has proved not to be effective in the control of FHB.

A better understanding of the effect that different agronomic practices might have on the development of FHB will help in devising a more effective strategy aimed at preventing its further spread and continued damage to the cereal industry in western Canada.

Few studies have looked at the impact of agronomic practices on FHB. In general, plot and survey studies on the effect of crop sequence have looked at the impact of a corn/wheat rotation on disease severity, the presence of *F. graminearum* in heads or grain, and/or DON levels (Cromey et al., 2002; Dill-Macky and Jones, 2000; Krebs et al., 2000; Miller et al., 1998; Obst et al., 1997; Schaafsma et al., 2001; Teich and Nelson, 1984; Teich and Hamilton, 1985; Yi et al., 2001). However, corn is not a common crop grown in rotation with wheat or barley in Saskatchewan. Many of the previous studies have also looked at the effect of tillage on FHB, FDK, or DON levels. However, these reports vary with respect to the impact that tillage and amount of crop residue had on disease levels, and in many cases showed no differences among tillage systems.

For the most part, the effect of herbicides on FHB has not been examined. Teich and Hamilton (1985) and Teich and Nelson (1984) reported no significant difference in Ontario between fields with or without herbicides applied to the wheat crop, but they did not identify the herbicides or the timing of application.

The objective of this study was to identify agronomic practices, or crop production factors (CPF), associated with the development of high FHB infection levels in eastern Saskatchewan, and to determine what factors would be necessary to change to prevent further damage caused by this disease. Reducing levels of FHB and preventing its further spread will help Saskatchewan producers remain competitive and protect market opportunities.

**Materials and Methods**

Number of fields sampled in southeastern Saskatchewan was 89 in 1999, 128 in 2000, 189 in 2001, and 253 in 2002. Most of the cultivars sampled belonged to three quality classes: 80% were CWRS, 13% Canada Western Amber Durum (CWAD), and about 7% Canada Prairie Spring (CPS).

At the mid-milk to early dough stage, heads were taken at random from each field. Percentage of heads with FHB symptoms (incidence) and severity (% of discolored spikelets) were determined visually. Discolored glumes were surface-sterilized and plated on modified PDA (Burgess et al., 1988) to confirm fungal infection, and for pathogen identification. An FHB index (% of heads infected X mean severity of infection)/100), was calculated for each wheat crop sampled.

Producers supplied information regarding the agronomic practices used on the crops sampled. The information included, among others, cultivar, seeding rate and date, crop history, N fertilizer use, herbicide use, and tillage management. This information was used to group the crops/fields into classes, or categories, based on CPFs. For cultivar susceptibility, wheat crops were categorized into susceptible and intermediate. Susceptible were rated as ‘poor’, and intermediate as ‘fair’ in Saskatchewan Varieties of Grain Crops (2003). For tillage system, fields were categorized according to the number of tillage operations they received in the previous three years: seven or more for conventional-till, one to six for minimum-till, and no passes for zero-till. Residue cover was not estimated for any field. For previously-grown crop, fields were categorized according to the crop, if any, grown the previous year: cereal, oilseed, pulse or summerfallow. For herbicide application, fields were categorized into two classes depending on whether they had received any applications of herbicide Groups 1, 2, 4 or 9 (GF) in the previous 18 months, or 3 years. The average number of GF applications that GF-treated fields received...
was 1.9 in the previous 18 months and 2.6 in the previous 3 years. With regards to N fertilizer use, crops grown on summerfallowed fields that had received more than 20 kg N ha\(^{-1}\), or crops grown after another crop receiving more than 35 kg N ha\(^{-1}\) were considered to have received ‘adequate’ N, whereas those that received less than these amounts were considered to have received ‘inadequate’ N fertilizer. No soil analysis was performed on any of these fields. For seeding rate, fields were considered to have been seeded at ‘recommended’ rates if these were 95 to 106 kg ha\(^{-1}\) for CWRS and CPS and 101 to 135 kg ha\(^{-1}\) for CWAD. Rates above these were considered as ‘high’ whereas those below were categorized as ‘low’.

In order to assess the relative contribution of each CPF to the total variance of FHB, we compared the ratios of the sum of squares of each CPF to the total corrected sum of squares from an analysis of variance (ANOVA) model where we included crop susceptibility, tillage system, previously-grown crop, and previous GF use. Although the variances of 1999 and 2002 were not homogeneous, this was not an obstacle to perform this particular ANOVA since we were only interested in estimating the contribution of each CPF to the total variance rather than their effects on the FHB index means.

The FHB data from each year were tested for normality using the Shapiro-Wilk test (Shapiro and Wilk, 1965). Because the data consisted of percentages, values were transformed using arcsine (Gomez and Gomez, 1984) prior to testing for normality. In addition, for the variables categorized into CPFs, a test of homogeneity of variances (Gomez and Gomez, 1984) was conducted for the arcsine-transformed data, and for the log transform of the arcsine transform. If this test determined that the variances of the categorized variables, or their transformations, were homogeneous across the CPF categories, the data were subjected to ANOVA using the CPF categories as class variables. For those variables where the data had heterogeneous variances, but the transformed data had homogeneous variances, ANOVAs were conducted on the transformed variables, and mean separations with one-degree of freedom contrasts were performed on the transformed data.

For those data whose variances were heterogeneous (1999 and 2002), the data were grouped into a ‘high’ and a ‘low’ FHB class based on the sample median as class separator. The effects of the CPFs on the distribution of observations into the high and low FHB classes were assessed with a chi-square test, under the assumption that if CPFs had no effect, the distribution of the observations into high and low classes would remain unchanged. For uniformity, chi-squares were also performed on the 2000 and 2001 data. If a cell in the contingency table had less than five observations, no chi-square test was performed for that specific comparison.

The proportion of fields that had received GF applications at any time in the previous 18 months or 3 years was highly dependent on the tillage system used by producers. For instance, the majority of fields under zero-till received applications of GF while very few fields under conventional-till management received any GF applications. This interdependence produced a non-uniform distribution of observations into the various categories, resulting in some of the cells having few observations (less than five), which compromised the integrity of the chi-square test. As a result, the effect of GF use (and other CPFs) on the proportion of fields in the high and low FHB class was analysed separately only for fields managed using minimum-till practices.

All statistical analyses were conducted with JMP 5.0.1.2 (SAS Institute, 2002). Statistical significance is indicated by: *, ** and *** (P<0.10, P<0.05 and P<0.01, respectively); ns: not significant at P>0.10.

Results and Discussion
Over the four years of this study, the most common Fusarium species isolated from infected wheat heads was *F. graminearum*, followed by *F. avenaceum*, *F. poae*, and *F. sporotrichioides*. Overall, the percentage of fields with FHB was highest in 2000 and 2001 (97-98%), and lowest in 1999 (71%) and
2002 (51%), whereas the mean FHB index was highest in 2001 (8.4%), intermediate in 2000 (2.7%) and lowest in 1999 (0.2%) and 2002 (0.4%).

In general, on a yearly basis from 1999 to 2001 the CPFs examined explained between 15 and 16% of the variance; in 2002 the model explained only 2% of the variance (Table 1). Annually, tillage systems and GF use accounted for a larger proportion of the variance than any other CPF, followed closely by previously-grown crop. Of all CPFs, GF use was the factor that accounted for a more consistent proportion of the variance every year, whereas the proportion explained by the other CPFs varied considerably from year to year, suggesting that weather conditions during the growing season determined to a large extent the relative importance of each CPF.

When all the data were pooled together across years, and we introduced a term to the model to represent the effect of growing season conditions (year factor), the model explained 38% of the variance, with the year term alone explaining 31.6% of the variance (Table 1). GF use, tillage system and previously-grown crop each accounted for nearly 2% of the variance, while cultivar susceptibility contributed less than 1% to the variance. The effects of herbicide Groups 1, 2 or 4, N fertilizer use, seeding rate and date had no significant effect on FHB in any year and therefore were not included in this analysis.

Table 1. Contribution of each crop production factor (CPF) to total observed variability.

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<thead>
<tr>
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<tbody>
<tr>
<td>Cultivar susceptibility</td>
<td>0.9</td>
<td>5.7</td>
<td>1.2</td>
<td>0.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Glyphosate use</td>
<td>5.4</td>
<td>3.1</td>
<td>7.4</td>
<td>0.4</td>
<td>2.1</td>
</tr>
<tr>
<td>Previously-grown crop</td>
<td>0.6</td>
<td>0.3</td>
<td>4.3</td>
<td>0.8</td>
<td>2.0</td>
</tr>
<tr>
<td>Tillage system</td>
<td>9.0</td>
<td>5.6</td>
<td>5.3</td>
<td>0.1</td>
<td>1.6</td>
</tr>
<tr>
<td>Environment</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>31.6</td>
</tr>
<tr>
<td>Whole model:</td>
<td>16.0</td>
<td>15.0</td>
<td>15.0</td>
<td>2.0</td>
<td>38.0</td>
</tr>
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1 contribution measured as (CPF sum of squares/corrected total sum of squares)*100.

The observation that the environment was the most important factor determining FHB development is supported by previous studies (e.g. Cromey et al., 2002; Schaarfsma et al., 2001).

The significance of the effects of the crop production factors (CPF) that affected FHB the most for each of the years is summarized in Table 2 for all fields sampled. Overall, the effects of the various CPFs on FHB were lower in years with high (2001) and low (1999 and 2002) disease pressure compared to a year with moderate (2000) disease pressure for this region of the Prairies.
Table 2. Significance of the effects of crop production factors (CPF) on the Fusarium head blight index of spring wheat sampled in southeastern Saskatchewan from 1999 to 2002.

<table>
<thead>
<tr>
<th>CPF</th>
<th>1999</th>
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<th>2001</th>
<th>2002</th>
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<tbody>
<tr>
<td>Cultivar susceptibility</td>
<td>ns¹</td>
<td>***</td>
<td>(*)</td>
<td>ns</td>
</tr>
<tr>
<td>Glyphosate use</td>
<td>*</td>
<td>**</td>
<td>**</td>
<td>*</td>
</tr>
<tr>
<td>Previously-grown crop</td>
<td>ns</td>
<td>*</td>
<td>ns</td>
<td>**</td>
</tr>
<tr>
<td>Tillage system</td>
<td>ns</td>
<td>(*)</td>
<td>**</td>
<td>ns</td>
</tr>
</tbody>
</table>

¹ ns=not significant, *, ** and ***, significant at P<0.10, 0.05 and 0.01, respectively, according to chi-square and ANOVA tests. Symbols in parenthesis indicate that only the ANOVA test was significant. Note that ANOVA tests were only conducted on the 2000 and 2001 data.

GF application in the previous 18 months (or 3 years) significantly increased disease levels every year (Table 2). Even though FHB levels were low, GF application was the only CPF in 1999, and one of only two CPFs in 2002, that significantly affected the FHB index. In addition, the fact that the GF effect was not confounded by that of other CPFs, such as tillage system, in 1999 and 2002, confirmed the observations made in the other years when disease levels were higher (2000 and 2001). These observations suggest that the effect of GF application on FHB was not affected by environmental conditions as much as the other CPFs.

Table 3. Significance of effects of crop production factors (CPF) on the Fusarium head blight index of spring wheat grown under minimum-till management in southeastern Saskatchewan from 2000 to 2002.

<table>
<thead>
<tr>
<th>CPF</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivar susceptibility</td>
<td>**¹</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Glyphosate use</td>
<td>**</td>
<td>***</td>
<td>*</td>
</tr>
<tr>
<td>Previously-grown crop</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

¹ ns=not significant, *, ** and ***, significant at P<0.10, 0.05 and 0.01, respectively, according to chi-square and ANOVA tests. Note that ANOVA tests were only conducted on the 2000 and 2001 data.

When only fields under minimum-till were analysed (Table 3), the effects of the previously-grown crop and cultivar susceptibility on the proportion of fields in the high FHB class were smaller than when all
crops were analysed together. However, the effect of GF application on FHB levels was greater in wheat crops under minimum-till than when all tillage systems were combined.

For 2000 and 2001, the years with the highest disease levels, the mean FHB index for all wheat crops grown in GF-treated fields was 75% higher than that of wheat grown in GF-untreated fields, while for fields under minimum-till management GF application increased the FHB index mean by 122%.

The design of this study allowed us to determine an association between GF use and increased levels of FHB in wheat crops, but did not permit us to determine the mechanism(s) by which previous GF applications increased FHB levels in spring wheat.

Previous studies have also reported a stimulatory effect of glyphosate on plant diseases, although none examined FHB in cereals or *F. graminearum*, the most important FHB pathogen in North America. A review by Levesque and Rahe (1992) discussed research indicating that herbicides can have a direct effect on various components of the soil microflora, such as plant pathogens, antagonists, or mycorrhizae, which can potentially result in increased or decreased incidence of plant disease. Pathogens able to infect weeds can also increase their inoculum potential after weeds have been sprayed with herbicides, which could subsequently affect host crops. Glyphosate application may indirectly stimulate pathogen invasion of weeds through root exudates after treatment. Controlling weeds that could act as hosts for pathogenic fungi could thus provide substrate for increased populations of pathogenic fungi.

*Fusarium* spp. are among several fungi that have been shown to act synergistically in causing the death of glyphosate-treated plants. Levesque et al. (1987) reported that glyphosate application increased root colonization of various treated weeds by *F. avenaceum* and *F. oxysporum*, and it also increased the propagule density of these *Fusarium* spp. in the soil. Levesque et al. (1992) reported that the efficacy of glyphosate on wheat seedlings depended on the synergistic action of these species and others in the soil.

Johal and Rahe (1984) and Rahe et al. (1990) suggested that poor emergence, establishment, and growth of crops planted soon after glyphosate treatment could be due to stimulation of pathogenic root fungal activity on treated plants. They concluded that the glyphosate-induced root colonization by *Fusarium* spp. and other pathogens was the cause, and not the result, of plant death following application of certain doses of glyphosate. Flax plants treated with glyphosate were also rapidly colonized by several species of fungi, including *F. culmorum* (Brown and Sharma, 1984).

Kawate et al. (1997) also suggested that weed control with glyphosate in the spring may provide *Fusarium* pathogens an energy source for survival and proliferation. *Fusarium* populations were greater in the rhizosphere soil from glyphosate-treated henbit than from untreated henbit. Pea planted in soil where henbit has been treated with glyphosate could be exposed to higher populations of *F. solani* f. sp. *pisi*. Similarly, glyphosate-treated quackgrass rapidly colonized by *F. culmorum* caused damage to the subsequent barley crop (Lynch and Penn, 1980).

Other reports have looked at disease levels in glyphosate-tolerant soybean and found that glyphosate-tolerant and -nontolerant cultivars responded similarly to infection by *F. solani* f. sp. *glycinus* (Sanogo et al., 2000; 2001) However, they also observed that after herbicide application a synergistic interaction with glyphosate occurred, and concluded that herbicide-induced stress could explain the significant increase in sudden death syndrome and pathogen isolation frequency following application of glyphosate and some other herbicides to glyphosate-treated soybean. On the other hand, Njiti et al. (2003) reported no effect of single glyphosate applications on development of sudden death syndrome in glyphosate-tolerant soybean.
In a study conducted in Missouri, Kremer (2003) reported that glyphosate applications on glyphosate-tolerant soybean at recommended rates resulted in increases in *Fusarium* populations on the roots and rhizosphere. These observations led them to conclude that glyphosate applications could cause increased disease levels in soybean and potential yield losses.

Glyphosate could also act directly on plants by inhibiting their phenolic metabolism which is necessary for the resistance mechanisms of plants, thus making them more susceptible to pathogenic organisms. Sub-lethal doses of glyphosate induced susceptibility to *F. oxysporum* f. sp. *radicis-lycopersici* in two resistant tomato cultivars whose root tissue was invaded by the pathogen soon after glyphosate treatment (Brammal and Higgins, 1988).

In *in vitro* studies, fungal growth of *F. graminearum* and *F. avenaceum* was stimulated when fungi were grown on media amended with glyphosate formulations (Hanson and Fernandez, 2003), whereas Krzysko-Lupicka and Orlik (1997) showed that *Fusarium* spp. grew out of soil suspensions only when these were plated on nutrient media in which glyphosate had been used as the sole source of C or P, but not on nutrient media alone.

In regards to tillage management systems, fields under minimum-till had in general the highest, or among the highest, disease levels in years with medium to high disease pressure, although this difference was significant only in 2000 and 2001 (Table 2). However, not all reduced tillage had the same effect on FHB. The use of zero-till did not result in an increase in FHB compared to conventional-till (2000) or had the lowest FHB levels (2001).

In Ontario, no significant differences among tillage methods were found by Miller et al. (1998) or Teich and Nelson (1984). In another FHB survey, Schaafsma et al. (2001) reported that DON levels in wheat under minimum-till were higher than in no-till or conventional-till, which would agree with our observations that minimum-till was most conducive to FHB development. On the other hand, Krebs et al. (2000) and Yi et al. (2001) reported the highest disease in no-till plots. Dill-Macky and Jones (2000) reported that FHB was lower in wheat grown in rotation with corn using moldboard plow compared to either chisel plowed or no-till plots. They attributed the lack of difference in FHB between chisel plow and no-till to the density and layering of residues in the latter which reduced residue to soil contact and might have affected the sporulation potential of the pathogen. The layering of the crop residues, poor fungal colonization of upper residues, leaching of antifungal compounds in the initial stages of residue decomposition, and/or other factors related to the microenvironment might explain the lower disease levels under zero-till than minimum-till observed in our study.

The even greater effect of GF application on FHB levels when fields under minimum-till were analysed separately suggest that the lower FHB index in wheat crops grown under zero-till than minimum-till management was not related to GF application, but to factor(s) intrinsic to zero-till and the lack of residue disturbance which appears to have impacted inoculum levels and/or its availability for head infection. In studies on the effect of tillage on tan spot on wheat, Fernandez et al. (1999b) also observed that crop residues in no-till had a lower density of pseudothecia of *Pyrenophora tritici-repentis* than those in conventional-till.

In general, the previously-grown crop did not affect FHB consistently across years, and this effect was only significant in 2000 and 2002 for all crops combined (Table 2). Previous comparisons of cereal and noncereal crops in rotation with wheat have not showed consistent differences in disease or mycotoxin levels either (e.g. Cromey et al., 2002; Dill-Macky and Jones, 2000; Obst et al., 1987; Schaafsma et al., 2001; Teich and Hamilton, 1985; Yi et al., 2001). The lack of a significant previously-grown crop effect could be due, at least partly, to noncereal residues harbouring FHB inoculum (e.g. Fernandez et al., 1992; Fernandez et al., 2003). *Fusarium graminearum* was also isolated from roots of live noncereal crops
grown in rotation with cereal crops in the FHB-affected area of eastern Saskatchewan (Fernandez, 2003), and is reported to be able to infect roots of noncereal crops under controlled conditions (Chongo et. al., 2001).

For cultivar susceptibility, overall the intermediate level of FHB resistance present in some wheat cultivars registered in western Canada resulted in lower FHB levels than in susceptible cultivars, although the difference between the intermediate and susceptible crops was only statistically significant in 2000 and 2001 for all crops combined (Table 2). This intermediate level of resistance appeared to be somewhat more effective under moderate (2000) than under high (2001) disease pressure for this area of the Prairies (Tables 2 and 3). As expected, differences in susceptibility to FHB among wheat cultivars did not play an important role under the low disease pressure experienced in 1999 and 2002.

Conclusions

- Differences in FHB levels in spring wheat from 1999 to 2002 indicate that the environment had the greatest effect on disease development in southeastern Saskatchewan.
- The effects of the various CPFs examined on FHB levels were more noticeable in a year with moderate disease pressure (2000) than in years with high (2001) or low (1999 and 2002) disease pressure for this region.
- Of the CPFs examined, previous applications of GF, tillage practice, previously-grown crop, and cultivar susceptibility had the largest effects on FHB levels. Neither herbicide Groups 1, 2 or 4, N fertilizer use, seeding rate nor date had a significant effect on disease levels.
- GF application in the previous 18 months (or 3 years) was significantly associated with higher FHB levels every year; it was the only CPF in 1999, and one of only two CPFs in 2002, that affected FHB, suggesting that the effects of this herbicide were not as influenced by environmental conditions as those of other CPFs. The relative effect of the other CPFs on FHB varied from year to year, and was significant in only one or two years. When wheat grown under minimum-till was analysed separately, GF application displayed an even greater effect on FHB than when all tillage systems were combined.
- Fields under minimum-till had the highest, or among the highest, disease levels in years with medium to high disease pressure. The use of zero-till management did not result in a significant increase in the FHB index compared to conventional-till practices, or had the lowest FHB levels. Lower FHB levels under zero-till than minimum-till systems were not related to GF application but to other factors intrinsic to zero-till management.
- The previously-grown crop, or summerfallow, did not affect FHB levels consistently across years.
- Lower FHB levels were observed in wheat crops with an intermediate level of resistance than in susceptible crops. These differences were not observed in years with low disease pressure (1999 and 2002).
- Even though FHB levels were considered low to high for this region of the Prairies, they were lower than in areas where FHB has occurred in epidemic proportions. It is not known if similar effects of GF on FHB would occur in environments different from the ones encountered in this study, less or more conducive to FHB development.
- Based on the statistically significant and consistent effect of previous GF application on FHB development in spring wheat throughout the four years of this study conducted in producers’ fields, further research is needed to elucidate the nature of the GF-FHB association and underlying mechanisms.
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