Sowing Density and Cultivar Effects on Pith Expression in Solid-Stemmed Durum Wheat

Kirby T. Nilsen, John M. Clarke, Brian L. Beres,* and Curtis J. Pozniak*

ABSTRACT

The wheat stem sawfly (WSS) (Cephus cinctus; Hymenoptera: Cephidae) is a destructive insect pest of spring, winter (Triticum aestivum L.), and durum wheat (Triticum turgidum L. var. durum) in North America. Sawfly larvae hatch from eggs deposited inside the stem, and their subsequent feeding damages vascular tissue, reducing photosynthetic capacity and grain yields. Growing solid-stemmed wheat cultivars that develop pith in the culm lumen is the most effective method to minimize yield losses. Recent work has focused on optimizing sowing densities to achieve maximum levels of pith expression and grain yields in common wheat; however, little research has been conducted on durum wheat. We investigated the influence of four sowing densities (150, 250, 350, 450 seeds m$^{-2}$) on pith expression to achieve maximum levels of pith expression and grain yields in common wheat; however, little research has been conducted on durum wheat. We investigated the influence of four sowing densities (150, 250, 350, 450 seeds m$^{-2}$) on pith expression in two newly released solid-stemmed durum cultivars, CDC Fortitude and AAC Raymore, and compared them to the solid-stemmed common wheat cultivar, Lillian. CDC Fortitude and AAC Raymore displayed consistently high levels of pith expression across environments and sowing densities, in contrast to Lillian, which produced only slightly more pith than the hollow-stemmed durum check cultivar, Strongfield. A yield drag often associated with high pith expression was not evident as CDC Fortitude and AAC Raymore produced grain yield similar to Strongfield. When averaged over cultivars, increasing sowing density had a positive effect on grain yield, but was negatively associated with stem solidness. Our findings suggest that, unlike with CWRS solid-stemmed cultivars, altering sowing density is not required to achieve effective sawfly resistance with CDC Fortitude and AAC Raymore.

THE WSS has been one of the most damaging insect pests of common and durum wheat across the northern Great Plains of North America for more than a century (Beres et al., 2011c). Severe yield losses occur when WSS larval feeding activity damages the inner stem tissue, which reduces flag leaf photosynthetic ability (Delaney et al., 2010), and damages vascular bundles impairing the flow of water and nutrients to the developing grain (Morrill et al., 1992). The greatest sawfly damage occurs in the southern parts of Alberta and Saskatchewan, southeastern Manitoba, and throughout Montana, North Dakota, South Dakota, and western Minnesota (Beres et al., 2011c).

The life cycle of the WSS has been reviewed in depth by Beres et al. (2011c). Briefly, the insect spends up to 10 mo of the year in its larval stage nested inside the wheat stem. In early spring, larvae will undergo pupation to reach the adult stage. Peak sawfly emergence generally occurs from 10 June to 10 July in western Canada (Beres et al., 2011b). Adult sawflies live for approximately 1 wk and usually mate shortly after emergence followed by oviposition within a few days. The female sawfly has a specialized saw-like ovipositor (hence “sawfly”) used to cut the wheat stem. During this process, the female will search for a suitable host plant in which to lay an egg, usually beginning at the upper internode of the developing wheat plant (Beres et al., 2011c). Females prefer succulent plants that are at early boot to anthesis stage with a large enough stem diameter suitable for oviposition (Holmes and Peterson, 1960). Eggs hatch approximately 1 wk after deposition, and larvae immediately begin feeding on the inner stem wall (Holmes, 1954). As the growing season progresses, larvae tunnel down through the stem, damaging vascular tissue disrupting the flow of nutrients and water to the developing wheat kernel (Morrill et al., 1992). At the end of the growing season, larvae will chew a notch around the inner perimeter of the stem at the base of the plant causing it to lodge. (Holmes, 1977). Due to the reduction in grain mass coupled with the fact that lodged stems are often

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Abbreviations: AAC, Agriculture and Agri-Food Canada; AC, Agriculture-Canada; CDC, Crop Development Centre; CWAD, Canada Western Amber Durum; CWRS, Canada Western Red Spring; GDD, growing degree days; NIL, near-isogenic line; WSS, wheat stem sawfly.
not picked up during harvest, yield losses attributed to the WSS can be as high as 30% (Beres et al., 2007).

One strategy to prevent yield losses from the WSS is to grow solid-stemmed cultivars that develop pith within the culm lumen (Clarke et al., 2002). The development of pith increases egg mortality through mechanical crushing, and can act as a barrier impeding larval growth and development inside the stem (Holmes and Peterson, 1961, 1962). Solid cultivars generally incur less damage from larval feeding, and have a lower proportion of stems cut at maturity (Sherman et al., 2015; Talbert et al., 2014). Surviving larvae collected from solid cultivars have lower body mass, size, and fecundity (Carcamo et al., 2005), although the presence of pith does not appear to significantly influence the overwintering mortality of the insect (Carcamo et al., 2011). Solid-stemmed cultivars are not immune to sawfly damage, but should be considered the starting point for an integrated pest management approach (Beres et al., 2013a). There are currently no registered pesticides available to control WSS because the insect spends most of its life cycle inside the wheat stem shielded from insecticide contact (Wallace, 1966). Adult sawflies emerge from infested stubble over a period of several weeks, making them difficult to control with a single chemical application, and multiple applications may be too costly to justify (Knodel et al., 2009).

In western Canada, peak sawfly emergence has been shown to coincide with growing degree days (GDD) (between 578 and 595 GDD), which could be used to predict the optimum time for chemical application (Beres et al., 2011a). Delaying seeding to avoid peak sawfly emergence time can substantially reduce damage, but this is not a practical strategy considering the short growing season on the Canadian prairies (Beres et al., 2007).

Attempts to select for host resistance to the wheat stem sawfly were initiated beginning in the early 1930s (Kemp, 1934). Most of the solid-stemmed common wheat cultivars are derived from the *T. aestivum* L. line S-615. The first S-615 derived cultivars were Rescue, Chinook, and Cypress (Larson and Macdonald, 1966). Currently registered S-615 derived solid-stemmed Canada Western Red Spring (CWRS) cultivars include AC Eaton (DePauw et al., 1994), AC Abbey (DePauw et al., 2000), and Lillian (DePauw et al., 2005). Pith expression in common wheat cultivars derived from S-615 can be quite variable and is heavily influenced by environmental factors. Sunlight intensity, the amount of precipitation, and days with measurable precipitation between the end of May to early July (Eckroth and McNeal, 1953; Holmes, 1984) all influence expression of pith under commercial sowing densities. Variable sowing densities have been investigated as one strategy to manipulate light penetration through the canopy to achieve maximum levels of pith expression. Because altering sowing densities can also influence other yield components, agronomic studies are essential to understand the best compromise for achieving maximum yield while maintaining adequate sawfly resistance. Similar studies have previously reported that pith expression in common wheat was inversely related to sowing density (Beres et al., 2011b, 2012), and was optimized at sowing densities of 250 to 350 seeds m⁻² (Beres et al., 2011b). In contrast, grain yield was positively related to sowing density, and was highest at sowing densities of 350 to 450 seeds m⁻² (Beres et al., 2011a).

The entire durum wheat growing region of North America falls within the geographical range of the WSS (Beres et al., 2011b), but to date no research has investigated the effect of sowing density on pith expression and components of grain yield in solid-stemmed durum wheat. Durum wheat displays superior sawfly tolerance over common wheat; and through greater straw strength, hollow-stemmed durum wheat cultivars are less prone to lodging than hollow common wheat once girdled and cut by larvae (Eckroth and McNeal, 1953). There are at least two main sources for stem solidness in durum wheat: (i) Golden Ball and (ii) Biodur. Recently, two new Biodur-derived solid-stemmed durum cultivars were registered in western Canada, AAC Raymore (Singh et al., 2014) and CDC Fortitude (Pozniak et al., 2015).

Pith expression in common wheat is controlled by the major quantitative trait loci (QTL) *Qst.ssub-3BL*, located on the long arm of chromosome 3B (Cook et al., 2004). Additional minor QTL have been localized to chromosomes 3D (Lanning et al., 2006) and 5D (Varella et al., 2015). Stem-solidness in durum wheat is controlled by a single dominant gene *Sst1* on chromosome 3BL (Houhmand et al., 2007) and its expression is relatively stable across environments (Clarke et al., 2002). Despite the proximity of *Sst1* to *Qst.ssub-3BL*, it remains unclear whether common wheat and durum wheat share the same gene for stem solidness.

The goal of this research was to investigate the effect of sowing density on pith expression and other agronomic traits in durum wheat to maintain the highest possible level of sawfly resistance without sacrificing grain yield potential. The research presented here tests the following hypotheses: (i) pith expression in durum wheat will decrease with increased sowing density, whereas grain yield will increase, (ii) pith expression in durum wheat will be more stable across environments than in common wheat, and (iii) solid-stemmed durum cultivars CDC Fortitude and AAC Raymore will be similar in grain yield to hollow check Strongfield.

**MATERIALS AND METHODS**

**Experimental Design**

Experimental field trials were established near Coaldale (AB, Canada), Lethbridge (AB, Canada), and Saskatoon (SK, Canada) during the 2012 to 2014 growing seasons (Table 1). The 2012 site near Saskatoon was located at the Goodale research farm. The 2013 field site at Goodale was lost due to early season flooding, therefore the location was moved to a field site near Kenaston (SK, Canada) in 2014. Cropping systems at all sites were conducted in a wheat-fallow scheme, except for the Kenaston site which was seeded into lentil stubble from the previous year. The experimental design for each location was a randomized complete block design (RCBD) with four replications, wherein treatments were arranged in a 4 × 5 factorial design testing the interaction between sowing density × cultivar. Five cultivars (CDC Fortitude, AAC Raymore, Strongfield, Golden Ball, Lillian) and four sowing densities (150, 250, 350, and 450 seeds m⁻²) were selected for this experiment. The cultivars were selected from the Canada Western Amber Durum (CWAD) and Canada Western Red Spring (CWRS) market classes, which included the hollow control Strongfield (Clarke et al., 2005), the solid-stemmed CWAD cultivars AAC Raymore (Singh et
al., 2014) and CDC Fortitude (Pozniak et al., 2015), the solid-stemmed CWRS cultivar Lillian (DePauw et al., 2005) and the solid-stemmed durum landrace Golden Ball (Clark et al., 1922). Seeds were treated with fungicide (Dividend XL RTA, Syngenta Crop Protection Canada) before sowing.

Experimental Measurements

Temperature and photoperiod were measured at each location using Hobo Pendent Temperature and light loggers (Onset Computer Corporation; part no. UA-002-XX). Sensors were attached to fiberglass stakes positioned above the level of the canopy and placed in each replication between two plots at the approximate center of each range. Supplemental long-term average monthly temperature and precipitation data were collected from Environment Canada (http://climate.weather.gc.ca/) for each location.

Plant density (plants m⁻²) was assessed in May–June, depending on location, by staking a 1-m section of two randomly selected rows within each plot. Spike densities (spikes m⁻²) were assessed between the end of July and early August using the same approach. The number of spikes per plant was calculated by dividing the total number of plants m⁻² by the number of spikes per m⁻². Plant heights (cm) were measured by taking an average of the whole plot after grain filling. Lodging ratings were recorded on a per plot basis before harvest using a 1 (no lodging) to 9 (completely lodged) scale.

Stem solidness ratings were recorded at physiological maturity by taking the average rating from the main stem of 10 to 25 plants selected randomly from each plot. Because the upper internodes of the plant were previously shown to be the most important for sawfly oviposition, stem internodes were labeled sequentially, beginning with internode 1 located closest to the spike of the plant, down to internode 5, located closest to the crown. Each stem was split longitudinally and each internode was assigned a rating according to the following scale; 1: Hollow stem- no pith development, 2: Minimal signs of pith development, may appear "cotton-like", 3: Large hollow tunnel in the stem, or, a huge cavity at a particular point in the internode, 4: Size of cavity equivalent to a pencil lead, or, some cavitation has occurred at a particular point in the internode, 5: Solid stem filled entirely with pith (Beres et al., 2012; DePauw and Read, 1982; Pozniak et al., 2015). Analysis of the stem solidness data was performed separately by internode, in addition to using a combined rating averaged across the entire stem.

Plots were harvested at maturity using a small plot combine to measure grain yield from which subsamples were collected to test for grain protein concentration (%), test weight (kg hL⁻¹) and seed mass (g 1000⁻¹). Grain protein was assessed using near infrared reflectance spectroscopy technology (Foss Decater GrainSpec, Foss Food Technology Inc., Eden Prairie, MN).
Normality of model residuals was assessed using the UNIVARIATE procedure of SAS 9.3. All interactions with random factors were considered as random within the mixed model. In cases where significance was declared, further testing was performed using Fisher’s least significant difference (LSD 0.05) test to characterize differences between treatment means using the PDMIX800 SAS macro (Saxton, 1998). Orthogonal contrasts were performed to test the relationship of sowing density with phenotypic expression of each trait. LSmeans for each treatment combination combined across environments were used to create a Pearson correlation coefficient matrix using the CORR procedure of SAS.

RESULTS

Monthly trends in temperature and light intensity were generally similar across testing environments, except for Lethbridge 2013 which received the highest light intensity, particularly during June and July (Table 1). Lower monthly average light intensities were recorded at Coalhurst (2014), and Lethbridge (2012 and 2014) compared to the other environments. There were, however, large variations in average weekly light intensity between environments, particularly between 5 and 10 wk after planting (Fig. 1). Growing season precipitation ranged from 191 to 359 mm (Table 1).

Stem solidness varied among cultivars at each internode, and when averaged over all stem internodes (Table 2). Pith expression was greatest in the two commercially registered solid-stemmed durum cultivars, CDC Fortitude and AAC Raymore, lowest in the hollow-stemmed cultivar Strongfield, and intermediate in Golden Ball and Lillian. All three solid-stemmed durum cultivars displayed the same pattern of pith expression across internodes, which tended to be greater in the upper internodes becoming progressively less in internodes closer to the crown (Table 2). In contrast, pith expression in Lillian and Strongfield was greater in lower internodes becoming less solid toward the spike. CDC Fortitude, AAC Raymore, and Golden Ball showed similar variation in pith expression between testing environments.

The relationship between sowing density and pith expression was linear and negative for each internode, and combined across internodes except for internodes one and five where no significant association was detected (Table 2). Maximum average stem pith expression was achieved with sowing densities below

![Fig. 1. Average weekly light intensity (lm m⁻² × 1000) recorded by hobo sensors in field testing environments during 2012 to 2014. Data are presented based on the number of weeks after planting.](image)

![Fig. 2. Influence of sowing density on (A) pith expression and (B) grain yield. Data were averaged across cultivars and testing environments 2012 to 2014. Means with the same letter grouping were not significantly different using Fishers LSD 0.05.](image)
450 seeds m\(^{-2}\) (Fig. 2A). Pith expression in internodes 2 and 3 was most negatively affected by increased sowing density (Table 2).

Cultivar and sowing density influenced grain yield but the interaction between cultivar and sowing density was not significant (Table 3). Grain yield of CDC Fortitude and AAC Raymore were not significantly different from Strongfield, and all three cultivars yielded significantly higher than Lillian or Golden Ball. Contrast analysis revealed that increasing sowing density had a positive effect on grain yield in all lines. Maximum levels of grain yield were observed at sowing densities above 150 seeds m\(^{-2}\) (Table 3, Fig. 2B).

The main effects of cultivar and sowing density were significant for all components of grain yield (spike density, stand density, test weight, protein content, seed mass, spikes per plant), but the cultivar \times sowing density interaction was not significant for any trait (Table 3). Spike density, test weight, and protein concentrations of CDC Fortitude and AAC Raymore were not significantly different from Strongfield. There were significantly higher plant stand densities observed in CDC Fortitude, AAC Raymore, Golden Ball, and Lillian than in Strongfield (Table 3). Strongfield, AAC Raymore, and Lillian had the highest spike densities, greater than in CDC Fortitude or Golden Ball. Similarly, Strongfield, Lillian, and AAC Raymore had a greater number of spikes per plant than CDC Fortitude or Golden Ball. Seed mass was greatest in Golden Ball, lowest in Golden Ball, and intermediate in Strongfield, AAC Raymore, and CDC Fortitude. Test weights were not significantly different between the four durum cultivars, all of which were significantly higher than in Lillian. Grain protein content was greatest in Lillian, lowest in Golden Ball, and intermediate in CDC Fortitude, AAC Raymore, and Strongfield (Table 3).

Contrast analysis revealed significant linear relationships between sowing density and all components of grain yield. Quadratic relationships were also observed for grain protein concentration and spikes per plant (Table 3). Increasing sowing density had a positive effect on plant stand density, spike density, and test weight; whereas the relationship was negative for seed mass, spikes per plant, and protein concentration (Fig. 3A–3F). Maximum test weights were observed at sowing densities above 150 seeds m\(^{-2}\). As expected, the responses among sowing density, plant stand density, and spike density displayed a pronounced linear relationship. Increasing sowing densities from 150 to 450 seeds m\(^{-2}\) resulted in over twice as many plants, and 27% more spikes per square meter. Seed mass and protein concentration were inversely related to sowing density, and both were greatest at sowing densities of 150 seeds m\(^{-2}\) (Fig. 3D, 3F).

Results from correlation analysis of stem solidness and agronomic traits are presented in Table 4. Average stem solidness was positively correlated with seed mass (\(r = 0.48\)), and negatively correlated with spike density (\(r = -0.51\)) and protein content (\(r = -0.51\)). There was no significant correlation between stem solidness and grain yield, plant density, test weight, plant height, lodging, or spikes per plant. Grain yield was strongly negatively correlated with lodging (\(r = -0.74\)) and plant height (\(r = -0.83\)), and positively correlated with test weight (\(r = 0.60\)). Plant density was strongly negatively correlated with the number of spikes per plant (\(r = -0.88\)) and positively correlated with spike density (\(r = 0.71\)). Seed mass was negatively correlated with protein content (\(r = -0.85\)) and spike density (\(r = 0.64\)), but positively correlated with lodging.

### Table 2. Stem solidness (1-solid to 5-solid) by internode and in response to sowing density across field testing environments during 2012 to 2014.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Treatment</th>
<th>Internode 1 (Spike)</th>
<th>Internode 2</th>
<th>Internode 3</th>
<th>Internode 4</th>
<th>Internode 5 (Crown)</th>
<th>Whole stem average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivar</td>
<td>CDC Fortitude</td>
<td>4.38</td>
<td>4.28</td>
<td>4.01</td>
<td>3.73</td>
<td>3.47</td>
<td>4.00</td>
</tr>
<tr>
<td></td>
<td>AAC Raymore</td>
<td>4.41</td>
<td>4.14</td>
<td>3.73</td>
<td>3.60</td>
<td>3.52</td>
<td>3.91</td>
</tr>
<tr>
<td></td>
<td>Golden Ball</td>
<td>4.05</td>
<td>3.91</td>
<td>3.69</td>
<td>3.54</td>
<td>3.42</td>
<td>3.73</td>
</tr>
<tr>
<td></td>
<td>Lillian</td>
<td>1.90</td>
<td>1.72</td>
<td>2.06</td>
<td>2.36</td>
<td>2.60</td>
<td>2.13</td>
</tr>
<tr>
<td></td>
<td>Strongfield</td>
<td>1.41</td>
<td>1.46</td>
<td>1.70</td>
<td>2.22</td>
<td>2.73</td>
<td>1.89</td>
</tr>
<tr>
<td>Sowing density,</td>
<td>Strongfield</td>
<td>0.081</td>
<td>0.071</td>
<td>0.072</td>
<td>0.072</td>
<td>0.097</td>
<td>0.063</td>
</tr>
<tr>
<td>seeds m(^{-2})</td>
<td>(0.05)§</td>
<td>0.158</td>
<td>0.139</td>
<td>0.142</td>
<td>0.142</td>
<td>0.19</td>
<td>0.124</td>
</tr>
<tr>
<td>150</td>
<td>LSD (&gt; F)</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>250</td>
<td>LSD (0.05)§</td>
<td>0.088</td>
<td>0.064</td>
<td>0.065</td>
<td>0.065</td>
<td>0.086</td>
<td>0.057</td>
</tr>
<tr>
<td>350</td>
<td>LSD (0.05)§</td>
<td>0.0998</td>
<td>0.0015</td>
<td>0.0002</td>
<td>0.0219</td>
<td>0.0894</td>
<td>0.0077</td>
</tr>
<tr>
<td>450</td>
<td>LSD (0.05)§</td>
<td>ns §</td>
<td>0.125</td>
<td>0.127</td>
<td>0.128</td>
<td>ns</td>
<td>0.112</td>
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<tr>
<td>Linear trend</td>
<td>Linear equation ((Y))</td>
<td>ns</td>
<td>-0.0008x + 3.33</td>
<td>-0.0009x + 3.30</td>
<td>-0.0006x + 3.27</td>
<td>ns</td>
<td>-0.0006x + 3.31</td>
</tr>
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<td></td>
<td>Regression value ((R^2))</td>
<td>ns</td>
<td>0.94</td>
<td>0.94</td>
<td>0.92</td>
<td>ns</td>
<td>0.96</td>
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<tr>
<td>Contrasts ((P &gt; F))</td>
<td>Linear</td>
<td>0.0194</td>
<td>0.0001</td>
<td>&lt;0.0001</td>
<td>0.0032</td>
<td>0.4643</td>
<td>0.0008</td>
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<tr>
<td></td>
<td>Quadratic</td>
<td>0.9792</td>
<td>0.6738</td>
<td>0.3692</td>
<td>0.679</td>
<td>0.8423</td>
<td>0.8002</td>
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<tr>
<td></td>
<td>Cubic</td>
<td>0.3977</td>
<td>0.4097</td>
<td>0.5489</td>
<td>0.4471</td>
<td>0.8435</td>
<td>0.5139</td>
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<tr>
<td>Cultivar \times</td>
<td>P (&gt; F)</td>
<td>0.3087</td>
<td>0.2164</td>
<td>0.354</td>
<td>0.2125</td>
<td>0.0889</td>
<td>0.1931</td>
</tr>
</tbody>
</table>

† SED: Standard error of the difference.
‡ LSD: Fisher’s protected least significant difference.
§ ns: Not significant.
Table 3. Components of grain yield by cultivar and in response to sowing density across field testing environments during 2012 to 2014.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Treatment</th>
<th>Grain yield Mg ha⁻¹</th>
<th>Plant stand density plants m⁻²</th>
<th>Spike density heads m⁻²</th>
<th>Seed mass g 1000⁻¹</th>
<th>Test weight kg hl⁻¹</th>
<th>Grain protein %</th>
<th>Spikes per plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivar</td>
<td>CDC Fortitude</td>
<td>3.31</td>
<td>192</td>
<td>241</td>
<td>41.1</td>
<td>77.6</td>
<td>14.0</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>AAC Raymore</td>
<td>3.24</td>
<td>187</td>
<td>248</td>
<td>43.8</td>
<td>77.5</td>
<td>14.4</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Golden Ball</td>
<td>2.59</td>
<td>181</td>
<td>219</td>
<td>46.3</td>
<td>77.3</td>
<td>13.3</td>
<td>1.4</td>
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<td></td>
<td>Lillian</td>
<td>2.86</td>
<td>182</td>
<td>278</td>
<td>36.4</td>
<td>76.2</td>
<td>15.9</td>
<td>1.7</td>
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<td>Strongfield</td>
<td>3.39</td>
<td>171</td>
<td>256</td>
<td>43.2</td>
<td>77.9</td>
<td>14.1</td>
<td>1.7</td>
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<td>SED †</td>
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<td>p &gt; F</td>
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<td>&lt;0.0001</td>
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<td>LSD (0.05) ‡</td>
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<td>0.30</td>
<td>12</td>
<td>35</td>
<td>2.0</td>
<td>1.1</td>
<td>0.43</td>
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<td>Sowing density, seeds m⁻²</td>
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<td>2.88</td>
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<td>218</td>
<td>42.9</td>
<td>77.0</td>
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<td>250</td>
<td>3.12</td>
<td>160</td>
<td>241</td>
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<td>41.9</td>
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<td>14.2</td>
<td>1.2</td>
</tr>
<tr>
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<td></td>
<td>0.081</td>
<td>16.2</td>
<td>12.1</td>
<td>0.37</td>
<td>0.13</td>
<td>0.09</td>
<td>0.07</td>
</tr>
<tr>
<td>p &gt; F</td>
<td></td>
<td>0.0052</td>
<td>&lt;0.0001</td>
<td>0.0004</td>
<td>0.0193</td>
<td>0.0074</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LSD (0.05) ‡</td>
<td></td>
<td>0.17</td>
<td>33</td>
<td>25</td>
<td>0.8</td>
<td>0.3</td>
<td>0.16</td>
<td>0.30</td>
</tr>
<tr>
<td>Linear trend</td>
<td>Linear equation (Y)</td>
<td>0.0009x + 2.80</td>
<td>0.4315x + 53.28</td>
<td>0.1996x + 188.54</td>
<td>-0.0039x + 43.34</td>
<td>0.0016x + 76.84</td>
<td>-0.0016x + 14.843</td>
<td>-0.0032x + 2.49</td>
</tr>
<tr>
<td>Regression (R²)</td>
<td></td>
<td>0.78</td>
<td>0.9997</td>
<td>0.99</td>
<td>0.94</td>
<td>0.94</td>
<td>0.91</td>
<td>0.90</td>
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<tr>
<td>Contrasts</td>
<td>Linear</td>
<td>0.0015</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0029</td>
<td>0.001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
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<tr>
<td>(P &gt; F)</td>
<td>Quadratic</td>
<td>0.166</td>
<td>0.8925</td>
<td>0.8243</td>
<td>0.4683</td>
<td>0.3516</td>
<td>0.04</td>
<td>0.032</td>
</tr>
<tr>
<td></td>
<td>Cubic</td>
<td>0.2258</td>
<td>0.996</td>
<td>0.5941</td>
<td>0.6398</td>
<td>0.9876</td>
<td>0.9367</td>
<td>0.758</td>
</tr>
<tr>
<td>Cultivar × sowing density p &gt; F</td>
<td></td>
<td>0.29</td>
<td>0.5474</td>
<td>0.5347</td>
<td>0.1031</td>
<td>0.8522</td>
<td>0.0697</td>
<td>0.1008</td>
</tr>
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</table>

† SED: Standard error of the difference.
‡ LSD: Fisher’s protected least significant difference.
Table 4. Pearson’s correlation coefficients testing the relationships between stem solidness, grain yield, and agronomic traits. All listed coefficients were significant at $P < 0.05$.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Average stem solidness</th>
<th>Spikes per plant</th>
<th>Protein content</th>
<th>Lodging</th>
<th>Height</th>
<th>Spike density</th>
<th>Test weight</th>
<th>Seed mass</th>
<th>Plant stand density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain yield</td>
<td>ns†</td>
<td>ns</td>
<td>ns</td>
<td>-0.74</td>
<td>-0.83</td>
<td>ns</td>
<td>0.60</td>
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<tr>
<td>Plant stand density</td>
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<td>-0.88</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.71</td>
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<tr>
<td>Seed mass</td>
<td>0.48</td>
<td>ns</td>
<td>-0.85</td>
<td>0.59</td>
<td>ns</td>
<td>-0.64</td>
<td>0.65</td>
<td></td>
<td></td>
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<tr>
<td>Test weight</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Spike density</td>
<td>-0.51</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Plant height</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.93</td>
<td></td>
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<tr>
<td>Lodging</td>
<td>ns</td>
<td>ns</td>
<td>-0.58</td>
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<td></td>
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<tr>
<td>Protein content</td>
<td>-0.51</td>
<td>0.44</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Spikes per plant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

† ns: No significant correlation.

Fig. 3. Influence of sowing density on components of grain yield: (A) plant stand density, (B) spike density, (C) spikes per plant, (D) seed mass, (E) test weight, and (F) protein content. Data were averaged across cultivars and testing environments 2012 to 2014. Means with the same letter groupings were not significantly different using Fishers LSD 0.05.
correlated with protein content \( (r = 0.59) \) and test weight \( (r = 0.65) \). Test weight was negatively correlated with protein content \( (r = -0.79) \). Spike density was negatively correlated with lodging \( (r = -0.58) \), whereas lodging was negatively correlated with protein content \( (r = -0.58) \).

**DISCUSSION**

This study showed that sowing density and cultivar influenced stem solidness, yield, and agronomic traits in durum and common wheat. Increasing sowing density negatively influenced pith expression and positively influenced grain yield in all cultivars. These results suggest sowing at densities of 350 seeds m\(^{-2}\) or less to maximize stem solidness. Female sawflies are known to preferentially select larger diameter stems for oviposition (Luginbill and McNeal, 1958). Because lower sowing densities are often associated with increased stem diameter, there may be an increased risk of stem cutting under extremely low sowing densities, which has been observed in some hollow-stemmed cultivars (Beres et al., 2011b). Therefore, sowing at densities closer to 350 seeds m\(^{-2}\) is recommended.

The solid-stemmed durum cultivars CDC Fortitude and AAC Raymore displayed superior stem solidness to Golden Ball and Lillian across all sowing densities. Both CDC Fortitude and AAC Raymore derive stem solidness from Biodur, which is a cultivar of German origin (Pozniak et al., 2015; Singh et al., 2014). A second source of stem solidness in durum wheat, Golden Ball, is also known to have pith expression superior to common wheat (McNeal, 1961). Golden Ball is of South African origin introduced to Canada but was later deregistered for its inferior pasta quality (Kemp, 1934; Knott, 1995). Although both sources were believed to carry the stem solidness gene \( SS1 \) (Houshmand et al., 2007), recent molecular evidence suggests the Biodur source may be different from Golden Ball based on differences in haplotype around the \( SS1 \) locus (Nilsen, unpublished data, 2015). Therefore, the improved solid stem quality of CDC Fortitude and AAC Raymore over Golden Ball might reflect genetic differences between sources, or could be a result of an accumulation of additional genetic factors conferring stem solidness throughout the breeding process.

The three solid durum cultivars had more pith development in the internodes closest to the spike, and became less solid toward the crown of the plant, in contrast to Lillian which had greater pith density in the lower internodes and became less solid toward the spike. This finding is in agreement with previous research, which found durum wheat to be more solid in the upper internode than common wheat cultivars derived from S-615. This difference was attributed to one or more genes on the D genome suppressing stem solidness in common wheat (Larson, 1959; McNeal and Wallace, 1967). The top internodes of the plant are the preferred location for sawfly oviposition (Holmes and Peterson, 1960), therefore increased pith in this area of the stem could play an important role in conferring an extra level of resistance against stem cutting or early stage larval feeding in durum wheat. The lower two internodes of the plant were previously shown to be the region where larvae feed and accumulate the bulk of their body mass before winter (Delaney et al., 2010); therefore Lillian could have a reduced level of resistance to early larval feeding in the upper internodes, but increased resistance against later stage larval feeding. Interestingly, the hollow-stemmed check Strongfield expressed some pith at the periphery of the inner stem walls, and tended to have a thicker outer stem wall than Lillian. Eckroth and McNeal (1953) found that hollow-stemmed durum wheat was more resistant to WSS than hollow-stemmed common wheat, but the reason for this increased resistance was unknown. The pith development coupled with the increased stem wall diameter in Strongfield may help to provide some resistance to stem cutting and could potentially slow larval movement inside the stem.

Grain yield was positively associated with increased sowing density in all cultivars, and stable yields were achieved at sowing densities above 150 seeds m\(^{-2}\). Increasing sowing density reduced tillering capacity in all lines as evidenced by a decrease in the number of spikes per plant. Interestingly, we did not see a corresponding increase in seed mass that was previously reported in common wheat (Beres et al., 2011b), which may have been due to a differential response between durum and common wheat. We did, however, find a positive relationship between sowing density and test weight. Taken together, these findings suggest that most of the yield advantage at higher densities must have come from a reduction in the number of tillers produced per plant coupled with an increase in the number of seeds produced per spike.

Grain protein content was negatively associated with sowing density across all lines, which was previously reported in common wheat (Beres et al., 2011b). In the present study, Lillian had the highest grain protein content of all the lines tested. This finding was expected, because Lillian carries the high protein gene \( GpB-1 \) and was the first solid-stemmed Canadian cultivar with yield and protein concentration similar to hollow-stemmed cultivars (DePauw et al., 2007).

CDC Fortitude and AAC Raymore yielded the same as the hollow-stemmed control Strongfield, which suggests that any yield penalty that may have been associated with the solid stem trait has been overcome through the breeding process. This was also reflected in the phenotypic correlation which revealed no significant relationship between stem solidness and yield. We did find negative correlations with protein content and spike density, and a positive correlation with seed mass, although these findings were probably more related to the differences between spring and durum wheat (lower protein, larger seed mass, and reduced tillering), rather than due to stem solidness directly. Historically, some producers of common wheat were reluctant to grow solid-stemmed cultivars because of a perception of a negative association between stem solidness and yield potential in the absence of sawfly infestation (Weiss and Morrill, 1992). One study found a negative correlation between stem solidness and yield in progeny derived from the cross Thatcher × Rescue (Rescue is a derivative of S-615) (McNeal et al., 1965). Conflicting studies have found no negative correlation (Cook et al., 2004; Hayat et al., 1995; Lanning et al., 2006; McNeal and Berg, 1979; Sherman et al., 2015). In the present study, we did not see a negative correlation between stem solidness and grain yield, which is in agreement with similar research performed in common wheat. However, further investigation to confirm this finding in durum wheat using near isogenic lines (NILs) is required. Taken together, these results demonstrate that producers should not be hesitant to seed solid-stemmed durum wheat regardless of sawfly pressure.
All lines showed variation in pith density across environments; however, pith expression in Lillian was more variable than the solid-stemmed durum cultivars tested. Wallace et al. (1973) suggest a minimum mean threshold stem solidness score of 3.75 to achieve effective resistance to the WSS. In this experiment, the solid-stemmed durum cultivars exceeded this level (average = 3.86) and thus should all have strong resistance across environments. In contrast, Lillian had only intermediate pith expression (average = 2.17), and in some environments was practically hollow stemmed. This finding was similar to (Beres et al., 2011b), who suggest differences in response to environment or limited genetic potential as a possible cause of reduced stem solidness in Lillian. Inconsistent pith expression has been an issue in many of the registered CWRS cultivars that were derived from the S-615 source (Beres et al., 2013b). Previous research showed that with S-615 derived cultivars, low light intensity or shading for a period of 7 d can negatively affect pith expression (Holmes, 1984). We performed further analysis to test for a correlation between average weekly light intensity between locations, and average stem solidness in Lillian (data not shown). Average light intensity (lux) received between 7 and 11 wk (42–77 d) after planting was strongly correlated with pith expression in Lillian (r = 0.93, p = 0.002), which corresponds roughly to the time between the start of stem elongation and the end of flowering. During these critical growth stages, we hypothesize that the formation of pith must be highly coordinated with internode elongation, which begins with the basal node, and progresses sequentially from node to node toward the top of the plant. Low light intensity during this period of plant development may inhibit cell division or growth within the pith tissue of the inner stem. Throughout most of the growing season of 2012, the Saskatoon and Lethbridge sites experienced the lowest light intensity of all testing environments, and subsequent pith expression in Lillian was poor. The highest average light intensity across testing environments was recorded at Lethbridge 2013, and corresponding pith expression in Lillian was the highest of any environment (average = 2.8). Taken together, these results suggest light intensity could be a major contributing factor toward the variation of pith expression in Lillian.

CONCLUSIONS

Modern durum cultivars CDC Fortitude and AAC Raymore, had stems that were significantly more solid than Golden Ball or the CWRS cultivar Lillian. When averaged over all cultivars, increasing sowing density tended to negatively influence pith expression. However, both new durum cultivars expressed superior stem solidness across all sowing density levels, above the recommended minimum threshold level to achieve effective sawfly resistance (Wallace et al., 1973). There was no negative association between stem solidness and grain yield in CDC Fortitude and AAC Raymore, as both produced grain yield similar to Strongfield. Yield was positively influenced by increasing sowing density in all cultivars, and stable yields were achieved at sowing densities greater than 150 seeds m⁻². These findings suggest that, unlike with CWRS solid-stemmed cultivars, it does not appear necessary to alter/layer sowing densities for CDC Fortitude and AAC Raymore, as the critical threshold for optimum pith expression was maintained at all sowing density levels (i.e., ≥3.75). Our results reinforce the important role that both solid-stemmed cultivars and proper agronomics can offer to a holistic IPM strategy for wheat stem sawfly management.

ACKNOWLEDGMENTS

The authors would like to acknowledge the technical support provided by Ryan Dyck, Ryan Babonich, Russell Lawrie, Hillory Smith, Heidi Lazorko, Vinh Tang, Chanshe Beierle, Steven Simmill, Warren Taylor, Dan Yangos, Regan Nielson, Jordana Hudak, Kim Ziegler, Valerie Leithoff, and Nicola Spencer. Funding provided by the Western Grains Research Foundation, Saskatchewan Ministry of Agriculture, Viterra, Genome Prairie, and Genome Canada is also greatly acknowledged. K. Nilsen acknowledges the financial support of the Robert P. Knowles Scholarship administered by the University of Saskatchewan.

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